Appendix A legends for supplementary methods

I. Correction constant for medication

Following correction constants were used to correct the values in the respective studies if participants reported lipid, anti-hypertensive, or blood pressure lowering medication use.[1,2]

Correction constant for lipid medication			
		mg/dl	mmol/l
HMG-CoA reductase inhibitors (statins)	HDL-C	- 2.3	- 0.06
	TG	+ 18.4	+ 0.208
Fibrates	HDL-C	-5.9	-0.153
	TG	+57.1	+ 0.645
Bile acid sequestrates	HDL-C	-1.9	-0.049
	TG	+ 0	+ 0
Correction constant for blood pressure			
		mmHg	
Anti-hypertensive or blood pressure lowering medication	DBP	+ 10	
	SBP	+ 15	
TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, DBP: Diastolic blood pressure, SBP: Systolic blood pressure			

II. Each study specific methods and acknowledgements

The Avon Longitudinal Study of Parents and Children (ALSPAC)

Design and study population

ALSPAC is a "transgenerational prospective observational study investigating influences on health and development across the life course".[3,4] Participants comprise a cohort of offspring born to pregnant women recruited in 1991-2 in Bristol, UK. Participants have been followed through a series of ongoing data collection waves involving questionnaires and clinical assessments. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). DNA methylation was measured in the peripheral blood of mothers and fathers approximately 18 years after the birth of the study child. The resulting profiles form part of the Accessible Resource for Integrated Epigenomics Studies[5] (ARIES, http://www.ariesepigenomics.org.uk/). All data are available by request from the Avon Longitudinal Study of Parents and Children Executive Committee (http://www.bristol.ac.uk/alspac/researchers/access/) for researchers who meet the criteria for access to confidential data. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Maternal smoking

Information on maternal smoking was self-reported in questionnaire by study participant in adulthood.

Metabolic Phenotypes

Metabolic phenotypes were measured during clinical examination. Peripheral blood samples were collected after an overnight fast for those attending before 14:00 or after a minimum 6 hour fast for those attending the clinic later in the day. Blood samples were obtained, centrifuged, separated and frozen at −80°C within 30 min. Plasma glucose was measured by the automated enzymatic (hexokinase) method. Lipids were measured by automated analyser with enzymatic methods. Weight and height were measured with the participants in light clothing and without shoes. Weight was measured to the nearest 0.1 kg with the use of Tanita scales. Height was measured to the nearest 0.1 cm with a Harpenden stadiometer. Waist circumference was measured twice to the nearest 1 mm at the midpoint between the lower ribs and the pelvic bone with a flexible tape. The mean of the two measures is used here. Blood pressure was measured while participants were lying down with the use of an Omron M6 monitor (Omron Healthcare UK Ltd, Milton Keynes, UK). Two readings of systolic and diastolic blood pressure were recorded on each arm, and the mean of these four readings was used here.

DNA methylation measurements

Genomic DNA was obtained from peripheral blood samples collected during the same clinic as for the metabolic phenotypes. It was bisulphite converted using the Zymo EZ DNA Methylation™ kit (Zymo, Irvine, CA), and DNA methylation was quantified using the Illumina HumanMethylation450 BeadChip according to manufacturer's instructions. During the data generation process a wide range of batch, variables were recorded in a purpose-built

laboratory information management system (LIMS). The LIMS also reported quality control (QC) metrics from the standard control probes on the 450k BeadChip for each sample. Samples failing QC were excluded from further analysis and the assay repeated. Sample QC and normalization was completed using the *meffil* package[6] in R version 3.2.0.

Covariates
Data on maternal smoking behaviour during pregnancy, age and gender were self-reported in questionnaire. Own smoking was available for only a subset of the participants, and DNA methylation at probe cg05575921 is known to be an accurate biomarker for smoking.[7] We therefore used the participants with own smoking information to obtain an optimal threshold for DNA methylation at cg05575921. Individuals with DNA methylation greater than 72% were considered smokers.

Cell type correction
Potential confounding effects of blood cell subtypes were estimated by the Houseman method.[8]

Batch correction
To account for batch effects in the data, probe intensities underwent a functional normalization approach[9] using the first 10 PCs of the Illumina 450K array control probes. This approach includes subset quantile normalization of the data and normal-exponential out-of-band background correction. In addition, surrogate variables[10] were generated and included in all regression models. The null model included all covariates (age, smoking, sex) and the full model additionally included outcome variables (maternal smoking during pregnancy, metabolic phenotypes).

Although ALSPAC maintains information for about 14,500 participant families, DNA methylation profiles are available for only 976 mothers and 554 fathers. Participants were selected for DNA methylation analysis based on having provided a blood sample at the clinic (approximately 18 years after the birth of the study child). Potential bias due to this subpopulation of ALSPAC was not handled in association tests.

Exclusion criteria
For metabolic phenotypes analyses, participants were excluded who were missing metabolic phenotype information (n=275). For the maternal smoking analyses, participants were excluded who were missing maternal smoking information (n=145).

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The Bogalusa Heart Study (BHS)
Design and study population
BHS is a long-term, community-based study of atherosclerosis beginning in childhood in a Black-White rural community in Louisiana, the United States.[11] Participants (n=968) who were examined during the 2006-2008 survey and had data on cardio-metabolic risk factors and DNA methylation quantified by the Infinium Human-Methylation450 BeadChip were included in the current study. All participants provided informed consent, while the study design and procedures as well as consent forms were approved by Institutional Review Board from Tulane University Medical Centre.

Metabolic Phenotypes
All measurements were obtained by trained study staff members who followed a standard protocol.[11] At each study visit, weight in light clothing without shoes was measured to the nearest 0.1 kg on a dedicated scale which was routinely calibrated; height was measured to the nearest 0.1 cm with a free-standing stadiometer. Height and weight were measured at least twice and the mean value of each of these was used to calculate BMI (calculated as weight in kilograms divided by height in meters squared). Intraclass correlation based on rescreening of about 10% randomly selected participants was 0.99 for both height and weight. Information on smoking and alcohol use was obtained by means of a staff administered standardized questionnaire.[12,13] Current smoking and drinking were defined as smoking at least one cigarette per day and consuming alcohol every day, respectively, during the prior 12 months. Blood pressure levels were measured using a mercury sphygmomanometer on the right arm of participants in a relaxed, sitting position. Arm length and circumference were measured to ensure use of the proper cuff size. Blood pressure levels were reported as the mean of 6 replicate readings. The measurements were conducted by 2 trained and randomly assigned observers. Study participants were instructed to fast for 12 hours before screening. Serum lipoprotein cholesterols and triglycerides were analysed using the Hitachi 902 Automatic Analyser (Roche Diagnostics, Indianapolis, IN) which employs a combination of heparin-calcium precipitation and agar-agarose gel electrophoresis procedures. The laboratory utilized is monitored for precision and accuracy by the Lipid Standardization and Surveillance Program of the Centres for Disease Control and Prevention, Atlanta, GA. Serum adiponectin levels were measured using a commercial radioimmunoassay kit (Linco Research, St Charles, MO). On the basis of blind duplicate determinations on ~10% of the study samples, the intraclass correlation coefficient of reliability was 0.93 for serum adiponectin levels.

Methylation measurements

Whole blood samples in the BHS were isolated for genomic DNA using the FlexiGene DNA kit (Qiagen). The Infinium HumanMethylation450K BeadChip (Illumina, San Diego, CA) was used for whole-genome DNAm analysis. All the samples were processed at the Microarray Core Facility Lab, University of Texas Southwestern Medical Centre, Dallas, TX, USA. For each subject, 750ng genomic DNA was bisulfite converted using the 96 well EZ DNAm kit (Zymo Research, Irvine, CA) according to manufacturer's instructions. The efficiency of the bisulfite conversion was confirmed by in-built controls on the 450K array. The methylation profile of each participant was measured by processing 4µl of bisulfite-converted DNA, at a concentration of 50ng/µl, on a 450K array. The bisulfite converted DNA was amplified, fragmented and hybridized to the array following the protocol. We scanned the arrays by using an Illumina iScan scanner, and then the raw methylation data was extracted using Illumina's Genome Studio Methylation Module. All the samples had call rates across all CpG sites on the array was greater than 99.8%. Data cleaning procedures were undertaken in Blacks and Whites, separately. The R package *Minfi*[14] was used for .IDAT processing, which allowed for quality control report, sample outliers identification, cell counts estimation, and annotation. Intensity files (*Red.idat and *Grn.idat) for each CpG site on the array were processed and converted into a β-value (analogous to the proportion of DNA methylated) between 0 (unmethylated) and 1 (fully methylated). Multi-dimensional scaling (MDS) plots were drawn for showing a 2-dimensional projection of distances between samples with different race and/or gender. All samples were classified into four specific groups, indicating correct information on self-reported gender and ethnicity. In addition, given the importance of cellular heterogeneity in DNAm, we estimated the relative proportions of six pure cell types (CD4+ and CD8+ T-cells, natural killer cells, monocytes, granulocytes, and b-cells) in each sample. The latest version of the Illumina Human Methylation 450k annotation data (version 1.2) was used in the current study to get the corresponding location information.

The R package *wateRmelon* was used for β-value normalization and quality control. For correction of systematic technical biases in the array (Type I and Type II probes), β-value normalization was performed by the "dasen" function, by which Type I and Type II intensities and methylated and unmethylated intensities were quantile normalized separately after backgrounds equalization.[15] The following criteria were used for filtering samples and probes: 1) samples having 1% of CpG sites with a detection p-value greater than 0.05; 2) probes having 5% of samples with a detection p-value greater than 0.05; 3) probes with beadcount less than 3 in 5% of the samples.

Data for a total of 448,829 CpG sites were retained each participant in the BHS discovery and replication samples. Further, the "champ.runCombat" function in the R package ChAMP applied a singular value decomposition method to correct for a variety of batch effects. The remnant batch effect was further adjusted in all analyses. Data cleaning and quality control procedures were similar in the primary replication sample (GHS), using the R package *Minfi*. Quantile normalization was performed with the "SWAN" function.[16]

Acknowledgements

The Bogalusa Heart Study is a joint effort of many investigators and staff members whose contribution is gratefully acknowledged. We especially thank the Bogalusa, LA school system, and most importantly, the children and adults who have participated in this study over many years.

BIOS consortium

BIOS consortium represent four cohorts with coordinated methylation measurements: the Cohort On Diabetes And Atherosclerosis Maastricht (CODAM), the Leiden Longevity Study (LLS), the Netherlands Twin Register study (NTR) and the Prospective Amyotrophic Lateral Sclerosis (ALS) study Netherlands (PAN).

Design and study population

LLS: The aim of the Leiden Longevity Study (LLS)[17] is to identify genetic factors influencing longevity and examine their interaction with the environment as a means to develop interventions to increase health at older ages. To this end, long-lived siblings of European descent were recruited together with their offspring and their offspring's partners, on the condition that at least two long-lived siblings were alive at the time of ascertainment. For men, the age criterion was 89 years or older; for women, the age criterion was 91 years or older. These criteria led to the ascertainment of 944 long-lived siblings from 421 families, together with 1,671 of their offspring and 744 partners.

NTR: The Netherlands Twin Register (NTR)[18–20] was established in 1987 to study the extent to which genetic and environmental influences cause phenotypic differences between individuals. To this end, data from twins and their families (nearly 200,000 participants) from all over the Netherlands are collected, with a focus on health, lifestyle, personality, brain development, cognition, mental health and aging.

CODAM: The Cohort on Diabetes and Atherosclerosis Maastricht (CODAM)[21] consists of a selection of 547 subjects from a larger population-based cohort.[22] Inclusion of subjects into CODAM was based on a moderately increased risk of developing cardio-metabolic diseases, such as type 2 diabetes and/or cardiovascular disease. Subjects were included if they were of European ancestry and over 40 years of age and additionally met at least one of the following

criteria: increased body mass index (BMI; >25), a positive family history for type 2 diabetes, a history of gestational diabetes and/or glycosuria, or use of antihypertensive medication.

PAN: The Prospective ALS study in the Netherlands is a population-based study that started in 2006, and is still ongoing. Main aim is to identify all patients with ALS in the Netherlands, and determine the incidence and prevalence through a capture-recapture design. As soon as a patient is identified, age and sex matched controls are being recruited through the general practitioner of the patient. Patients and controls are being sent the same questionnaire in order to gather data on life style, family history, smoking, diet, medical history and past environmental exposures through occupations and hobbies. Also, blood is drawn after broad consent to enable further studies in DNA and serum.[23]

Maternal smoking
Not applicable

Metabolic Phenotypes
Height and weight were measured during examination and converted to body mass index (BMI). Triglycerides and high density lipoprotein cholesterol were measured after a fasting period of 12 h for NTR, CODAM and PAN; for LLS non-fasted lipids were measured.

Methylation measurements
The generation of genome-wide DNA methylation data is previously described [8]. Briefly, 500 ng of genomic DNA was bisulphite modified using the EZ DNA Methylation kit (Zymo Research) and hybridized on Illumina 450K arrays according to the manufacturer's protocols. Data was generated by the Human Genotyping facility (HugeF) of ErasmusMC, The Netherlands.

Covariates
Data on smoking status, age and gender were self-reported in questionnaire.

Cell type correction
Potential confounding effects of blood cell subtypes were estimated by the Houseman method[8] in CODAM and PAN, whereas percentage of measured cell counts (granulocytes, lymphocytes and monocytes) were used for LLS and NTR.

Batch correction
Sample quality control was performed using MethylAid. Probes with a high detection P value (> 0.01), probes with a low bead count (< 3 beads), and probes with a low success rate (missing in > 95% of the samples) were set to missing. Samples containing an excess of missing probes (> 5%) were excluded from the analysis. Subsequently, per cohort, imputation was performed to impute the missing values. To account for batch effects in the data, functional normalization, as implemented in the minfi package,[9] was used per cohort using the first 5 PCs.

Exclusion criteria
Participants on medication, and non-overlap samples between phenotypes and DNA methylation were excluded. For NTR, single twins were used.

Acknowledgements (BIOS consortium)
NTR, LLS, CODAM and PAN
Samples were contributed by the Leiden Longevity Study (http://www.leidenlangleven.nl), the Netherlands Twin Registry (http://www.tweelingenregister.org), the CODAM study (http://www.carimmaastricht.nl/), and the PAN study (http://www.alsonderzoek.nl/). We thank the participants of all aforementioned biobanks and acknowledge the contributions of the investigators to this study, especially Aaron Isaacs, René Pool, Marian Beekman, P. Mila Jhamai, Michael Verbiest, H. Eka D. Suchiman, Marijn Verkerk, Ruud van der Breggen, Jeroen van Rooij, Nico Lakenberg, Jan Bot, Patrick Deelen, Irene Nooren, Martijn Vermaat, Dasha V. Zhernakova, René Luijk, Freerk van Dijk, Wibowo Arindrarto, Szymon M. Kielbasa, and Morris A. Swertz. This work was carried out on the Dutch national e-infrastructure with the support of SURF Cooperative.

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Estonian Genome Centre, University of Tartu (EGCUT)

Design and study population

The Estonian Genome Centre at the University of Tartu (EGCUT) is a population based biobank which comprises health, genealogical and 'omics' data of close to 52,000 individuals ≥ 18 years of age, closely reflecting the age distribution in the adult Estonian population.[24] Participants of the EGCUT have been recruited by clinicians at their offices or data collectors at recruitment offices of the EGCUT. A computer assisted personal interview was completed for each participant, including personal data (place of birth, place(s) of living, nationality etc.), genealogical data (family history, three generations), educational and occupational history and lifestyle data (physical activity, dietary habits, smoking, alcohol consumption, quality of life). The collection of blood samples and the data is conducted according to the Estonian Human Gene Research Act and all participants have signed a broad informed consent. Individuals for this study were selected from a subgroup of "healthy" individuals who have been re-contacted for a second time point interview.

Metabolic Phenotypes

Height, weight and waist circumference were measured during the interview by data collector at recruitment offices of the EGCUT. Systolic and diastolic blood pressure were measured three times and average of the measurements was used. Serum high density lipoprotein cholesterol (HDL-C) and triglyceride levels were determined by enzymatic colorimetric methods using a cobas c 501 clinical chemistry analyser (Roche). Glucose levels were analysed by an enzymatic reference method using hexokinase on the Cobas c 501 module (Roche).

Methylation measurements

DNA from whole blood was extracted by the salting-out method using 10 M ammonium acetate. The DNA was precipitated in isopropanol, washed in 70% ethanol, and finally resuspended in 1X TE buffer. The purity and concentrations of the DNA samples were measured by NanoDrop ND-1000 spectrophotometry. 500 ng of genomic DNA was treated with sodium bisulphite using the EZ DNA Methylation Kit (Zymo Research Corporation) according to the manufacturer's instructions. DNA methylation analysis was performed using the Infinium Human Methylation 450K BeadChip (Illumina).

Quality control and normalization of the raw DNA methylation data was done following the pipeline by Lehne *et al.*[25] Samples with call rate < 95% using detection p-value threshold of $p < 10^{-16}$ were excluded. Illumina background correction was applied to all intensity vales. Intensity values were divided into six categories and quantile normalized separately. Normalized intensity values were used to calculate beta-values used in the analyses. CpG sites on both X and Y chromosomes were excluded.

Covariates

Age, gender, smoking status (as reported in the questionnaire) was used as covariates in the model.

Cell type correction

Potential confounding effects of blood cell subtypes were estimated by the Houseman method as implemented in the *minfi* package.

Batch correction

To adjust for technical bias, principal component analyses was performed on control probe intensities and 30 PCs were used in the models.

Exclusion criteria

Out of 318 samples, five were excluded having call rate > 95% and one were excluded based on the methylation patterns of sex chromosomes.

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Italian cardiovascular section of EPIC (EPICOR Study)

Design and study population

The Italian cardiovascular section of EPIC (EPICOR study)[26] is a case-cohort study nested in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Italy cohort. The EPIC-Italy cohort comprises about 50,000 participants[27]

enrolled between 1992 and 1998, who provided at enrolment a detailed dietary and lifestyle questionnaire and a blood sample that was stored in liquid nitrogen for later use. The EPIC cohort is regularly followed up for the occurrence of cancers and other non-communicable diseases of adulthood. Four EPIC-Italy centres (Turin, Varese, Naples, and Ragusa) provided samples to EPICOR. The whole EPICOR study comprises more than 1,500 subjects with cardiovascular outcomes such as myocardial infarction (MI), acute coronary syndrome, ischemic cardiomyopathy, coronary or carotid revascularization, ischemic- or haemorrhagic stroke. Within the EPICOR cohort, a subset of 584 subjects (292 MI cases and 292 matched controls) was analysed as a nested case-control study and underwent DNA methylation analysis and whole genome genotyping. All volunteers signed an informed consent form at enrolment in the respective studies. EPICOR study complies with the Declaration of Helsinki principles and conforms to ethical requirements The EPIC study protocol was approved by Ethics Committees of the International Agency for Research on Cancer (Lyon, France), as well as by local Ethical Committees of the participant centres. The EPICOR study was approved by the Ethical Committee of the Italian Institute for Genomic Medicine (IIGM, formerly Human Genetics Foundation-Torino, HuGeF, Turin, Italy).

Maternal smoking
No information on maternal smoking was evaluated in the study.

Metabolic Phenotypes
Information on metabolic phenotypes were measured during clinical examination (HDL, triglycerides, glucose, measured as mmol/L). Height and weight were converted to body mass index (BMI, measured as Kg/m^2). Waist Circumference (centimetres) was measured by nurse from the point midway between the costal margin and the iliac crest and recorded to one decimal place in centimetres. Systolic and diastolic blood pressures (mmHg) were measured twice with mercury sphygmomanometer in sitting position. Correction constant for lipid medication and for blood pressure was applied.
Methylation measurements
DNA methylation was measured in DNA from WBCs collected at subject enrolment into EPIC and stored in liquid nitrogen. Genomic DNA was extracted from 400ul buffy coat from whole blood stored in liquid nitrogen at sample recruitment by an automated on-column DNA purification method (QIAsymphony instrument and QIAsymphony DNA Kits, QIAGEN GmbH, Germany), according to manufacturer's standard protocols. DNA integrity was checked by an electrophoretic run in standard TBE 0.5X buffer on a 1% low melting agarose gel (Sigma-Aldrich GmbH, Germany); DNA purity and concentration were assessed by a NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific Inc.). Five hundreds of genomic DNA were bisulphite converted (EZ-96 DNA Methylation-Gold Kit, Zymo Research Corporation) according to manufacturer's protocol. The methylation status of more than 485,000 individual CpG loci at a genome-wide resolution was assessed by the Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, CA, USA) according to standard manufacturer protocols. Functional normalization for Whole-genome methylation data quality control (QC) and normalization procedures was performed: a total of 292 matched case-control pairs (584 subjects) passed QCs and 484683 CpG sites passed QCs and were retained for further analyses.

Covariates
Age, gender and Participant's smoking.

Cell type correction
Potential confounding effects of blood cell subtypes were estimated by the Houseman method.

Batch correction
To account for batch effects in the data, beta values underwent a functional normalization approach using the first 20 PCs of the Illumina 450K array control probes.

Exclusion criteria
No subjects were excluded.

Acknowledgements
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The ESTHER Cohorts (ESTHER-subset-a and ESTHER-subset-b)
Design and study population
ESTHER: The ESTHER study is an ongoing population-based cohort study conducted in the federal state of Saarland, Germany.[28] In brief, 9,949 older adults (50-75 years) were recruited by their general practitioners (GPs) during routine health check-ups (offered every two years to people older than 35 years in the German healthcare system) between 2000

and 2002, and followed up thereafter. During the baseline enrolment, epidemiological data (including socio-demographic characteristics, lifestyle factors, and history of major diseases) were collected via a standardized self-administered questionnaire completed by participants and via additional reports from participants' GPs, and biological samples (blood, stool, urine) were obtained and stored at −80 °C. Two subsets of ESTHER participants were selected for DNA methylation assessment in the baseline blood samples: Subset_a consists of 1,000 participants consecutively enrolled during the first 3 months of recruitment; Subset_b consists of 864 participants selected for a case-cohort design for mortality analysis.[29] The study was approved by the ethics committees of the University of Heidelberg and of the Medical Association of Saarland. All participants provided written informed consent.

Maternal smoking
NA.

Metabolic Phenotypes
Information on metabolic phenotypes, including weight and height (for BMI calculation), systolic and diastolic blood pressure, high density lipoprotein cholesterol (HDL-C), and fasting glucose were measured by general practitioners during the health check-ups, i.e. baseline enrolment. Serum triglycerides were determined using a high-performance liquid chromatography method calibrated with the Synchron LX multicalibrator system (Beckman Coulter, Galway, Ireland) in a central lab, where all biological samples were processed during the baseline recruitment.

Methylation measurements
DNAm in whole blood was quantified using the Infinium HumanMethylation450K BeadChip (Illumina.Inc, San Diego, CA, USA). In brief, 1.5 mg DNA (allocated in 96-well format with three random duplicate samples in each format as quality controls) was bisulphite converted, and 200 ng bisulphite-treated DNA was applied to the 450K BeadChips following the manufacturer's instruction. Raw data pre-processing and initial quality control was carried out following the CPACOR pipeline.[25] Probes with detection p-value>0.01 were removed before quantile normalization, which was applied following stratification of the probe type into 6 categories according to probe type and colour channel, using the R package limma.[30] Sample call rate threshold and CpG call rate threshold both were 95%.

Covariates
Data on age, gender, and smoking status were self-reported in questionnaire.

Cell type correction
Leukocyte composition was estimated using Houseman et al.'s algorithms.[8]

Batch correction
A principle component analysis (PCA) was performed for the positive control probes, and the first 30 control probe PCAs were included in the regression model as technical covariates.
Exclusion criteria
NA. (No missing value for all included 8 CpGs)

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The Cooperative Gesundheitsforschung in der Region Augsburg (Cooperative Health Research in the Augsburg Region) F4 (KORAF4)

Design and study population
Our study population consisted of participants from the KORA (Kooperative Gesundheitsforschung in der Region Augsburg) study,[31] which has been collecting clinical and genetic data from the general population in the region of Augsburg, Germany for more than 20 years. The cohort investigated in this paper is the S4 study, carried out in 1999-2001. The follow-up (F4) took place in 2006-2008. At both assessments, participants completed a lifestyle questionnaire and underwent standardized examinations with blood samples taken, as described elsewhere.[31,32] The KORA studies were approved by the Ethics Committee of the Bavarian Medical Association (Bayerische Landesärztekammer; S4: #99186, F4: #06068) and were conducted according to the principles expressed in the Declaration of Helsinki. All study participants gave their written informed consent.

Maternal smoking
Not applicable.

Anthropometric and Metabolic Phenotypes
Anthropometric measurements were taken in light clothing as described previously.[33,34] Height was measured to the nearest 0.1 cm; weight to the nearest 0.1 kg. Body mass index was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the level midway between the lower rib margin and the iliac crest. Systolic and diastolic blood pressures were measured for seated individuals by trained and certified personnel. Three measurements were taken at least three minutes apart using the HEM-705CP (Omron Healthcare GmbH); the mean of the last two measurements was used.
A fasting blood sample was obtained from all study participants. Blood was collected without stasis, cooled at 4–8°C and shipped to the laboratory on refrigerant packaging within 4-6 hours. For the KORA S4 study, lipid levels and blood glucose were determined as described in[33]. Total cholesterol was measured using the Boehringer CHOD-PAP (Roche Diagnostics, Mannheim, Germany) and HDL-cholesterol using the phosphotungstic acid method (Boehringer, Mannheim). Triglycerides were measured with the Boehringer GPO-PAP assay. Blood glucose was measured using the Gluco-quant hexokinase method (Roche Diagnostics).
For the KORA F4 study, glucose and lipid levels were determined as described in[35,36]. Specifically, total cholesterol, LDL-C, HDL-C, and triglyceride levels were measured using the cholesterol-esterase, ALDL, AHDL Flex, and TGL Flex methods (CHOL Flex, Dade-Behring, Germany), respectively. Blood glucose was analysed using the GLU Flex hexokinase method (Dade-Behring).

Methylation measurements and normalization
1802 individuals from the F4 study were randomly selected for DNA methylation measurement. 1535 S4 individuals were randomly selected from those having methylation data measured at the F4 time point. For the S4 and F4 studies DNA methylation was measured in the whole blood of the participants using the Infinium HumanMethylation450K BeadChip. The bisulphite conversion and genome-wide methylation assessment were performed as previously described.[37] The F4 methylation was measured in 2012 and S4 was measured in two batches in 2014.
Normalization of the methylation data was conducted following the CPACOR pipeline[25], beginning with exclusion of 65 single-nucleotide polymorphism markers and background correction using the R package minfi version 1.6.0. Probes were set to NA if the detection p-value ≥ 0.01 or number of beads ≤ 3. Samples were excluded if the detection rate was ≤ 0.95. Quantile normalization using the R package limma version 3.16.5 was then performed on the signal intensities divided by colour, probe type and methylated/unmethylated subtype (6 categories total: type-I M red, type-I U red, type-I M green, type-I U green, type-II red, type-II green). The methylation of a given cytosine was first calculated as a β-value, the ratio of the methylated signal intensity to the sum of the methylated and unmethylated signal intensities. Samples were excluded if they had less than 95% valid β-values across all CpG sites (0 in S4, 75 in F4).

Covariates
Data on age, gender, smoking status and medication use were self-reported in the questionnaire.

Cell type correction
Potential confounding effects of blood cell subtypes were estimated by the Houseman method.[8]

Technical correction
To account for technical effects, the first 30 principal components of the non-negative control probes were used as covariates in the regression models: for the cross-sectional analysis the principal components were derived from the F4 control probes alone; for the longitudinal analysis the principal components were calculated based on the combined control probe data of S4 and F4 together.

Exclusion criteria
For F4, 26 individuals were eliminated in the cross-sectional analysis, and 3 in the longitudinal analysis, due to missing values in more or more of the covariates. For S4, 2 individuals were eliminated for this reason.

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The Lifelines Deep (LLD)
Design and study population
The Lifelines study follows over 167,000 participants over a 30-year period. Initially, Lifelines participants were recruited through general practitioners in the three provinces in the northern part of the Netherlands: Groningen, Friesland and Drenthe. General practitioners invited everyone aged between 25 and 50. These individuals were then asked to invite their family members (parents, partner, children, parents-in-law). This approach has resulted in a three-generation cohort. Other interested individuals could also register for participation in the Lifelines study. Between 2006 and 2013, over 167,000

participants registered. Lifelines DEEP includes an intensively examined subpopulation of the Lifelines cohort in the Netherlands.[38] In this unique sub-cohort, we included 1539 participants aged 18 years and older. The LifeLines DEEP study was approved by the ethics committee of the University Medical Centre Groningen. All participants signed an informed consent prior to enrolment.

Maternal smoking

NA

Metabolic Phenotypes

Metabolic phenotypes were measured during clinical examination from April to August 2013

DNA methylation measurements

Initially, 1539 participants were included in the LifeLines DEEP study. Of these participants, 78 dropped out: 51 did not complete the second visit to the LifeLines location in time and 27 withdrew from participation. In total, 1461 individuals completed the LifeLines DEEP study. From these participants, we collected additional blood for genetics, methylation and transcriptomics analyses (n=1387); exhaled air for analysis of volatile organic compounds (n=1425); and faecal samples for microbiome and biomarker assessment (n=1248). Moreover, 1176 GI symptoms questionnaires were returned. For 81% (n=1183) of the participants, we collected all three biomaterials: additional blood, exhaled air and faeces (see online supplementary figure S1). For 11.5% (n=168) of the participants, we collected additional blood and exhaled air, and for 4.4% (n=65) of the participants, we collected exhaled air and faeces. For 3.1% of the participants, we only have additional blood (2.5%, n=36) or exhaled air (0.6%, n=9).

Genomic DNA was obtained from peripheral blood samples collected in EDTA tubes during the same clinic as for the metabolic phenotypes. DNA methylation was quantified using the Illumina HumanMethylation450 BeadChip according to manufacturer's instructions. In short, 500 ng of genomic DNA was bisulfite modified and used for hybridisation on Infinium HumanMethylation450 BeadChips, according to the Illumina Infinium HD Methylation protocol. Sample QC, quantile-normalization and the analyses were completed according to the pipeline by Lehne et al.[25] in R version 3.2.0.

Covariates

Age, gender, was used as covariates in the model.

Cell type correction

Potential confounding effects of blood cell subtypes were estimated by the Houseman method as implemented in the *minfi* package

Batch correction

To adjust for technical bias, principal component analyses was performed on control prone intensities and 30 PCs were used in the models.

Exclusion criteria

Participants with self-reported Crohn's disease, ulcerative colitis and celiac disease were excluded from this analysis.

Acknowledgements

We would like to thank the LifeLines participants and the staff of the LifeLines study site, Groningen, for their collaboration.

The London Life Science Population study (LOLIPOP)

Design and study population

LOLIPOP[39,40] is a prospective population study of ~28K Indian Asian and European men and women, recruited at age 35–75 years from the lists of 58 General Practitioners in West London, UK, between May 1, 2002, and Sept 12, 2008. Indian Asians had all four grandparents born on the Indian subcontinent (India, Pakistan, Sri Lanka, or Bangladesh); Europeans were of self-reported white ancestry. At enrolment, all participants completed a structured assessment of cardiovascular and metabolic health, including anthropometry, and collection of blood samples for measurement of fasting glucose, lipid profile, and complete blood count with differential white cell count. Personal and family history were collected, including smoking habit. The LOLIPOP study is approved by the National Research Ethics Service (07/H0712/150) and all participants gave written informed consent at enrolment.

Maternal smoking

Information on maternal smoking was not available.

Metabolic Phenotypes

At enrolment, participants were seen between 0800 h and 1200 h, after an overnight 8-h fast, for collection of fasting blood samples for measurement of complete blood count, glucose, and lipid concentrations. Aliquots of whole blood

were stored at -80C for extraction of genomic DNA. Height and weight were recorded, and body mass index (BMI) was calculated as weight in kilograms by height in metres. Waist circumference was measured from the point midway between the costal margin and the iliac crest. Systolic and diastolic blood pressure was obtained using the OMRON 705CP monitor. The participant was in sitting position with the right forearm supported on the table, after 5 minutes of rest, before the blood pressure measurements were taken. Three readings were taken 1 minute apart, and average of the measurements was used.

Methylation measurements
DNA methylation was quantified in bisulphite-converted genomic DNA from baseline whole blood samples, using the Illumina HumanMethylation450 array.[25,41,42] Quantification was on a scale of 0-1, in which 1 represents 100% methylation.

Covariates
We performed principal component analysis on the signal intensities for the positive control probes. The first 20 control-probe principal components were included as covariates in the models to remove technical biases. Other covariates included age, gender, and imputed WBC subsets.

Cell type correction
White-blood-cell types were estimated using a previously described method.[8] In that paper, 500 CpG sites showing the most pronounced cell-type-specific methylation levels were reported in an experiment based on purified cells. Of these, 473 CpGs were available on the 450K array we used. Following the proposed procedure and using the R code provided (R function projectWBC), we used these 473 CpG sites to infer white-blood-cell proportions (that is, the proportion of granulocytes, monocytes, B cells, $CD4^+$ T cells, $CD8^+$ T cells and natural killer cells) in our samples. These proportions were then used as covariates in the models to avoid cell-type confounding.

Batch correction
Raw signal intensities were retrieved using the function readIDAT of the R package minfi v.1.6.0 from the Bioconductor open source software, followed by background correction with the function bgcorrect.illumina from the same R package. To reduce non-biological variability between observations, data were quantile normalized with the function normalizeQuantiles of the R package limma v.2.12.0 from Bioconductor, separately in six probe categories based on probe type and colour channel.
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The Northern Finland Birth Cohorts (NFBC1966 and NFBC1986)
Design and study population
NFBC1966: The Northern Finland Birth Cohort 1966 is a prospective follow-up study of children from the two northernmost provinces of Finland.[43] 96% of all woman in this region with expected delivery dates in 1966 were recruited though maternity health Centres (12,058 live births). All individuals still living in northern Finland or the Helsinki area (n = 8,463) were contacted and invited for clinical examination. A total of 6007 participants attended the clinical examination at the participants' age of 31 years. DNA was extracted from blood samples given at the clinical examination (5,753 samples available)[44].The subset with DNA is representative of the original cohort in terms the major environmental and social factors known to influence the tested trait. An informed consent for the use of the data including DNA was obtained from all subjects.
In 2012, all cohort members with known address in Finland were sent postal questionnaires and an invitation to a clinical examination at age of 46 years. DNA methylation at 31 years was measured for 807 randomly selected subjects that attended the clinical examination and completed the questionnaire at both 31 and 46 years. DNA methylation at 46 years was measured for 766 subjects for whom DNA methylation at 31 years was also available. For DNA methylation marker calling we used a detection *P* value threshold of $<10^{-16}$ A call rate filter of 95% was applied to the all autosomal Illumina probes yielding 459378 probes for association testing. 67 samples were excluded due to low marker call rate (<95%). 7 samples were excluded for gender inconsistency; one sample for globally outlying DNA methylation values (1st PC score of the DNA methylation values outside mean +/- 4SD).

NFBC1986: The Northern Finland Birth Cohort 1986 consists of 99% of all children, who were born in the provinces of Oulu and Lapland in Northern Finland between 1 July 1985 and 30 June 1986. 9,203 live-born individuals entered the study[45]. At the age of 16, the subjects living in the original target area or in the capital area (n=9,215) were invited to participate in a follow-up study including a clinical examination. 7344 participants attend the study in year 2001/2002, of which 5654 completed the postal questionnaire, the clinical examination and provided a blood sample[46]. DNA was extracted from all 5654 blood samples. An informed consent for the use of the data including DNA was obtained from all subjects. DNA methylation was recoded on Illumina HumanMethlation450K array for 546 randomly selected subjects. 24 technical replicates were excluded. 18 samples did not reach a call rate of >95% applying a detection P-value filter of 10-16 .We excluded 7 samples with gender inconsistency, no sample was outlying from the overall data structure (1st PC score of the DNA methylation values outside mean +/- 4SD). DNA methylation data of 517 samples with 466290 autosomal probes (call rate filter 95%) each were used for this analysis.

Maternal smoking
Information on maternal smoking was self-reported in questionnaire by mother during pregnancy.

Metabolic Phenotypes
Information on metabolic phenotypes were measured during clinical examination. During examination, height and weight were measured to an accuracy of 0.1 cm and 0.1 kg and converted to body mass index (BMI). Waist Circumference was measured by nurse from the point midway between the costal margin and the iliac crest and recorded to one decimal place in centimetres. Blood samples were taken after an overnight fast from the subjects in the morning. All samples were analysed at Oulu University Hospital laboratory. Analyses were conducted within 24 hours for serum high density lipoprotein cholesterol (HDL-C) and triglycerides and determined by enzymatic methods using a Hitachi 911 Clinical Chemistry Analyser. Serum fasting glucose assay samples were stored at −20°C and analysed within 7 days of sampling by a glucose dehydrogenase method (Granutest 250, Diagnostica Merck). Systolic and diastolic blood pressure were measured twice with mercury sphygmomanometer in sitting position from the right arm, after 15 minutes of rest by trained nurses. Two readings were taken 2 min apart, and average of the measurements was used.
Methylation measurements
Methylation of genomic DNA was quantified using the Illumina HumanMethylation450 array (NFBC 1966 at age 31, NFBC 1986) or Illumina EPIC array (NFBC 1966 at age 46) according to manufacturer's instructions. Bisulphite conversion of genomic DNA was performed using the EZ DNA methylation kit according to manufacturer's instructions (Zymo Research, Orange, CA).

Covariates
Data on adult smoking status, age and gender were self-reported in the questionnaire in both cohorts.

Cell type correction
Potential confounding effects of blood cell subtypes were estimated by the Houseman method.[8]

Batch correction
To account for technical variation including batch effects, principal component analysis was carried out for array control probes, and the first 30 principal components were included in the regression model as technical covariates.

Exclusion criteria
NFBC1966: For the main analysis we excluded n=75 participants: 0 twins, and participants with missing information on methylation (n=75).
NFBC1986: For the main analysis we excluded n=51 participants: 0 twins, and participants with missing information on methylation (n=51).

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The West Australian Pregnancy Cohort (RAINE) Study

Design and study population
The RAINE Study enrolled pregnant women ≤18 weeks gestation (1989-1991) through the antenatal clinic at King Edward Memorial Hospital and nearby private clinics in Perth, Western Australia.[47,48] Detailed clinical assessments were performed at birth (n=2,868) and the children followed up at multiple time points including at 17 years of age, when a

blood sample was taken, and waist/hip circumference, skinfold thickness and abdominal (subcutaneous and visceral) adipose thickness measurements made.[49] DNA methylation was recoded on Illumina HumanMethlation450K array for 1192 subjects obtained at the 17 year old follow up. 58 technical replicates were excluded. DNA methylation beta-values were normalized using Beta-mixture quantile dilation (BMIQ) as described by Teschendorff et al.[50] We excluded three samples that were identified as outliers and one sample for sex inconsistency. The Human Ethics Committees of King Edward Memorial Hospital and Princess Margaret Hospital approved all protocols.

Maternal smoking
Information on maternal smoking was self-reported in questionnaire by mother during pregnancy.

Metabolic Phenotypes
Detailed information on measures of metabolic phenotypes collected in RAINE are provided in detail elsewhere.[51,52] Briefly, blood pressure was measured with an automatic device (Dinamap Vital Signs Monitor 8100, Dinamap XL Vital Signs Monitor or Dinamap ProCare 100; GE Healthcare) after 5 min rest and using the appropriate cuff size. Six readings were recorded, and the average value was calculated after excluding the first reading. Height and weight were measured with light clothing and without shoes. Height was measured with Holtain Infantometer and Stadiometer (to the nearest 0.1 cm), and weight was measured on Wedderburn Scales (to the nearest 100 g). Fasting venous blood samples were drawn for DNA and biochemical analyses. Serum insulin, glucose, total cholesterol, high density lipoprotein (HDL)-cholesterol, LDL-cholesterol and triglycerides were measured in the PathWest Laboratory at Royal Perth Hospital as described previously.[51,52]
Methylation measurements
Methylation of genomic DNA was quantified using the Illumina HumanMethylation450 array and carried out at the Centre for Molecular Medicine and Therapeutics (http://www.cmmt.ubc.ca). Bisulphite conversion was prepared from whole blood cells by standard phenol: chloroform extraction and ethanol precipitation.

Covariates
Data on maternal age, income, parity and maternal smoking during pregnancy were assessed by questionnaires at 18 and 34 weeks pregnancy. We used plate and plate position as technical batch variables.

Cell type correction
Potential confounding effects of blood cell subtypes were estimated by the reference-based Houseman method.

Batch correction
To account for potential batch effects, plate and row number included in all statistical models as covariates.

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The Rotterdam Study (RS) –RSIII-1 and RSII-3_III-2

Study population
This study was performed among participants of the prospective population-based Rotterdam Study. In 1989, all 10,275 residents aged 55 years or older in Ommoord, a suburb of Rotterdam, were invited to participate in the study. In 2000, the Rotterdam Study was extended by including 3,011 participants that moved to Ommoord or people who turned 55 (RS-II). The third cohort was formed in 2006 and included 3,932 participants 45 years and older (RS-III). Participants have been re-examined every 3-4 years and have been followed up for a variety of diseases. The Rotterdam Study has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants in the present analysis provided written informed consent to participate and to obtain information from their treating physicians. A more detailed description of the Rotterdam Study can be found elsewhere.[53] For this study, we used data from the first visit of RSIII (a random set of 731 individuals), and another non-overlapping set of the third visit of RS-II and the second visit of RS-III (719 individuals) with both DNA methylation and smoking data.

Metabolic Phenotypes
Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared whereas waist circumference was measured at the level midway between the lower rib margin and the iliac crest, with participants in standing emptied out pockets, breathing out gently
position without heavy outer garments and with. Information on current and past smoking behaviour was acquired from questionnaires. Blood lipids and glucose levels were measured using automatic enzymatic procedures (Roche Diagnostics

GmbH, Mannheim, DE). Blood pressure was measured in the sitting position on the right arm and calculated as the mean of two measurements using a random-zero sphygmomanometer. White cell counts were used when available or estimated leukocyte proportions (B-cells, CD4+ T-cells, CD8+ T-cells, granulocytes, monocytes and NK-cells) were calculated as described by Houseman and implemented in the minfi package in R.[8,14]

DNA methylation data
DNA was extracted from whole peripheral blood (stored in EDTA tubes) by standardized salting-out methods. Genome-wide DNA methylation levels were measured using the Illumina Human Methylation 450K array.[54] In short, samples (500ng of DNA per sample) were first bisulphite-treated using the Zymo EZ-96 DNA-methylation kit (Zymo Research, Irvine, CA, USA). Next, samples were hybridized to the arrays according to the manufacturers' protocol. The methylation proportion of a CpG site was reported as a beta-value ranging between 0 (no methylation) and 1 (full methylation). The data pre-processing was additionally performed in both datasets using an R programming pipeline based on the pipeline developed by Tost & Toulemat,[55] which includes additional parameters and options to pre-process and normalize methylation data directly from idat files. 11,648 probes at X and Y chromosomes were excluded to avoid gender bias. The raw beta values were then background-corrected and normalized using the DASEN option of the WateRmelon R-package.[15]

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The Study of Health in Pomerania (SHIP-Trend)
Design and study population
SHIP-Trend: The Study of Health in Pomerania (SHIP-TREND) is a longitudinal population-based cohort study in West Pomerania, a region in the northeast of Germany, assessing the prevalence and incidence of common population-relevant diseases and their risk factors. Baseline examinations for SHIP-Trend were carried out between 2008 and 2012, comprising 4,420 participants aged 20 to 81 years. Study design and sampling methods were previously described.[56] The medical ethics committee of the University of Greifswald approved the study protocol, and oral and written informed consents were obtained from each of the study participants.

Methylation measurements and exclusion criteria
DNA was extracted from blood samples of n=160 SHIP-Trend participants to assess DNA methylation using the Illumina HumanMethylationEPIC BeadChip array. Samples were randomly selected based on availability of multiple OMICS data taking the distribution of cardiovascular risk factors (hypertension, obesity, type II diabetes, smoking, and lipid levels) into account. The samples were taken between 07:00 AM and 04:00 PM, and serum aliquots were prepared for immediate analysis and for storage at -80 °C in the Integrated Research Biobank (Liconic, Liechtenstein). Processing of the DNA samples was performed at the Helmholtz Zentrum München. Preparation and normalization of the array data was performed according to the CPACOR workflow[25] using the software package R (www.r-project.org). The array idat files were processed using the minfi package. Probes that had a detection p-value above background (sum of per-array methylated and unmethylated intensity values based p-value ≥1E-16) were set to missing. Methylation beta values were calculated as proportion of methylated intensity value on the sum of methylated+unmethylated+100 intensities. Arrays with observed technical problems (±4SD outside control probe intensity mean) during steps like bisulphite conversion, hybridization or extension, as well as arrays with mismatch between sex of the proband and sex determined by the chr X and Y probe intensities were removed from subsequent analyses. Additionally, only arrays with a call rate ≥ 95% were processed further resulting in 248 samples with methylation data on 865,859 sites available for subsequent analyses.

Metabolic Phenotypes
To account for potential confounding effects due to blood cell composition, blood cell subtypes were estimated by the Houseman method[8] and included in the association model. Additionally, the first four principal components of the control probe intensities obtained by the CPACOR workflow were included in the model to account for technical factors such as batch effects. Details on assessment of the metabolic phenotypes and covariates used in this analysis are provided within the SHIP cohort design.

Young Finns Study (YFS)

Design and study population

The Cardiovascular Risk in Young Finns Study Cohort is a population-based follow-up study on cardiovascular risk factors in Finland.[57] The study comprises of six cohorts representing general population, born in 1962, 1965, 1968, 1971, 1974 and 1977 from five cities with university hospitals in Finland (Helsinki, Kuopio, Oulu, Tampere and Turku). This study is primarily based on 2063 participants (45% men, mean age 41.8 years) from 2011 follow-up. DNA was extracted from venous blood samples after an overnight fast and serum was separated, aliquoted and stored at −70°C until analysis. The study has been approved by the Joint Commission on ethics of the Turku University and the Turku university Central Hospital. The study has been conducted according to the guidelines of the Declaration of Helsinki, and informed consent was obtained from all participants. DNA methylation was measured in a subsample of randomly selected 192 individuals. We used a detection P-values threshold of <10-16. A call rate filter of 95% was applied to the all autosomal Illumina probes yielding 473864 probes for association testing. Two samples were excluded for gender inconsistency, one sample for methylome age discrepancy and two for failing quality control based on log median intensity in both the methylated (M) and unmethylated (U) channels.

Maternal smoking

Information on maternal smoking was not available.

Metabolic Phenotypes

Information on metabolic phenotypes was measured during clinical examination. During examination, height and weight were measured to an accuracy of 0.1 cm and 0.1 kg and converted to body mass index (BMI). Waist Circumference was measured by nurse from the point midway between the costal margin and the iliac crest and recorded to one decimal place in centimetres. Blood samples were taken after an overnight fast from the subjects in the morning. All samples were analysed at Tampere University Hospital laboratory.

Serum triglycerides was determined by the enzymatic glycerol kinase–glycerol phosphate oxidase method (Triglyceride reagent, Beckman Coulter Biomedical, Ireland) and analysis was performed within 24 hours. High density lipoprotein (HDL)-cholesterol levels were determined by the enzymatic cholesterol esterase–cholesterol oxidase method (Cholesterol reagent, Beckman Coulter Biomedical) after precipitation of low density lipoprotein (LDL) and very low density lipoprotein with dextran sulphate–Mg2+.[58] Serum glucose assay samples were stored at −20°C and analysed within 7 days of sampling by the enzymatic hexokinase method (Glucose reagent, Beckman Coulter Biomedical). Systolic and diastolic blood pressures were measured thrice with mercury sphygmomanometer in sitting position from the right arm, after 15 minutes of rest by trained nurses. Average of the measurements was used.

Methylation measurements

Methylation of genomic DNA was quantified using the Illumina HumanMethylation450 array according to manufacturer's instructions. Bisulphite conversion of genomic DNA was performed using the EZ DNA methylation kit according to manufacturer's instructions (Zymo Research, Orange, CA).

Covariates

Data on the used covariates were self-reported in questionnaire.

Cell type correction

Potential confounding effects of blood cell subtypes were estimated by the Houseman method.[8]

Exclusion criteria

For the main analysis, we excluded n=6 participants: two based on sex discrepancy, one based on age discrepancy and three based on log median intensity quality control.

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