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Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

Metabolic Age Based on the BBMRI-NL ^{1}H-NMR Metabolomics Repository as Biomarker of Age-related Disease

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BACKGROUND: The blood metabolome incorporates cues from the environment and the host's genetic background, potentially offering a holistic view of an individual's health status.

METHODS: We have compiled a vast resource of proton nuclear magnetic resonance metabolomics and phenotypic data encompassing over 25 000 samples derived from 26 community and hospital-based cohorts.

RESULTS: Using this resource, we constructed a metabolomics-based age predictor (metaboAge) to calculate an individual's biological age. Exploration in independent cohorts demonstrates that being judged older by one's metabolome, as compared with one's chronological age, confers an increased risk on future cardiovascular disease, mortality, and functionality in older individuals. A web-based tool for calculating metaboAge (metaboage.researchlumc.nl) allows easy incorporation in other epidemiological studies. Access to data can be requested at bbmri.nl/samples-images-data.

CONCLUSIONS: In summary, we present a vast resource of metabolomics data and illustrate its merit by constructing a metabolomics-based score for biological age that captures aspects of current and future cardiometabolic health.

Key Words: aging ■ cardiovascular disease ■ data science ■ metabolomics

Chronological age is an important risk factor for virtually all types of common disease, including diabetes mellitus type 2, cardiovascular disease, and many forms of cancer.[1] Moreover, chronological age is often used as an important criterion on which clinical treatment decisions in older adults are based. Yet, especially in the elderly, chronological age is a poor representative of an individual's intrinsic biological age, including the susceptibility to disease and resilience to treatment.[2] Hence, novel biomarkers are required that give additional information about the disparity between chronological and biological age, that is, whether individuals are biologically older and potentially more vulnerable than their peers.

A range of multimarker algorithms has been developed to serve as indicators of biological age. Examples are those based on physiological deterioration of organ systems

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The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCGEN.119.002610.

For Sources of Funding and Disclosures, see page 546.

Circulation: Genomic and Precision Medicine is available at www.ahajournals.org/journal/circgen

Nonstandard Abbreviations and Acronyms

^{1}H-NMR	Proton Nuclear Magnetic Resonance
5CV	5-Fold-Cross-Validation
BBMRI-NL	Biobanking and Biomolecular Resources and Research Infrastructure the Netherlands
BMI	body mass index
LOBOV	Leave-One-Biobank-Out-Validation
PROSPER	Prospective Study of Pravastatin in the Elderly at Risk
LLS-SIBS	Leiden Longevity Study - nonagenarian siblings

from the second[3] or third[4] decade onward or those based on combined health deficits in later life, the so-called frailty indices.[5,6] Others have exploited large quantities of highly standardized molecular data, for example, DNA methylation data, to train the so-called clock algorithms[7–9] that allow one to calculate an omics-based age. The difference between an individual's actual chronological age and the estimated methylation age was for instance shown to associate with mortality.[10] Interestingly, when compared, each of these omics-based biological age indicators appeared to mark unique aspects of ageing,[11,12] giving ample incentive for the development of other, possibly complementary omics-based indicators of biological age. While several large epidemiological studies on the blood metabolome have revealed many age-associated changes in metabolite levels, as determined by either mass spectral analyses,[13] or proton nuclear magnetic resonance (^{1}H-NMR),[14] to date, only studies of a fairly limited size have been used to construct a metabolomics clock.[15]

METHODS

Data are available upon request. Please visit bbmri.nl/samples-images-data and fill out and sign the data access request and code of conduct forms to request the data in this manuscript. Application complaints with ethical and legal legislations will be reviewed by the Dutch Biobanking and Biomolecular Resources and Research Infrastructure the Netherlands (BBMRI-NL) board for overlap with other ongoing projects before access is granted.

Included studies have been approved by their respective local medical ethical committees, and all participants gave informed consent for study participation. Detailed Methods are available in the Data Supplement.

RESULTS

BBMRI-NL Resource

We present a novel, well-standardized ^{1}H-NMR blood-based metabolomics dataset encompassing over 25 000 samples collected by the Dutch Biobanking and BioMolecular Resources and Research Infrastructure derived from 26 community- and hospital-based cohorts (Figure 1; Table II in the Data Supplement for cohort descriptions; data available upon request at BBMRI-NL: bbmri.nl/samples-images-data). We have used these data to construct a metabolomics-based clock (predictions made available as web resource; metaboage.reasearchlumc.nl; see Methods for instructions) and show that the difference between chronological age and metabolomic age captures aspects of cardiometabolic health.

Deriving a Metabolomics-Based Score for Biological Age

A metabolomics predictor for chronological age was trained and evaluated (Document III in the Data Supplement) using 56 of 226 most reliable and independent[16] metabolomic variables (Document II in the Data Supplement; Table III in the Data Supplement), derived from 24 cohorts (Figure 1). Two biobanks missing a metabolomic variable were omitted (Methods). In addition, PROSPER and LLS_SIBS were left out from training the metabolomic age predictor and used to independently explore the predictive value of the obtained indicator of biological age. With use of the data of the remaining 22 biobanks comprising 18 716 samples (9680 men and 10 036 women), a linear model was trained with the 56 metabolomic variables to estimate chronological age (Tables IV and V in the Data Supplement; Methods). A 5-Fold-Cross-Validation (5CV; Methods; Document III in the Data Supplement) scheme was used for randomly splitting the data in training (80%; 15 208 samples) and test (20%; 3802 samples) sets for an unbiased training and evaluation of the models. In addition, model performances were evaluated using Leave-One-Biobank-Out-Validation (LOBOV; Methods; Document III in the Data Supplement) to simulate the scenario of applying the trained model to a completely unseen dataset. While LOBOV results displayed more variation in prediction performances compared with 5CV, they overall showed good agreement between predicted and chronological age for all analyzed biobanks (Document III in the Data Supplement). The age-independent part of the difference between the estimated metabolomic age and chronological age (Figure 1C), hereafter referred to as ΔmetaboAge, may reflect for each individual the disparity between their biological and chronological age (Methods). Consequently, a high ΔmetaboAge indicates a relatively old blood metabolome for a given chronological age.

Associations of metaboAge With Cardiometabolic Risk Factors

In subsequent analyses, we explored which aspects of biological age are marked by ΔmetaboAge. First, we investigated whether ΔmetaboAge correlates with established clinical risk factors for cardiometabolic disease

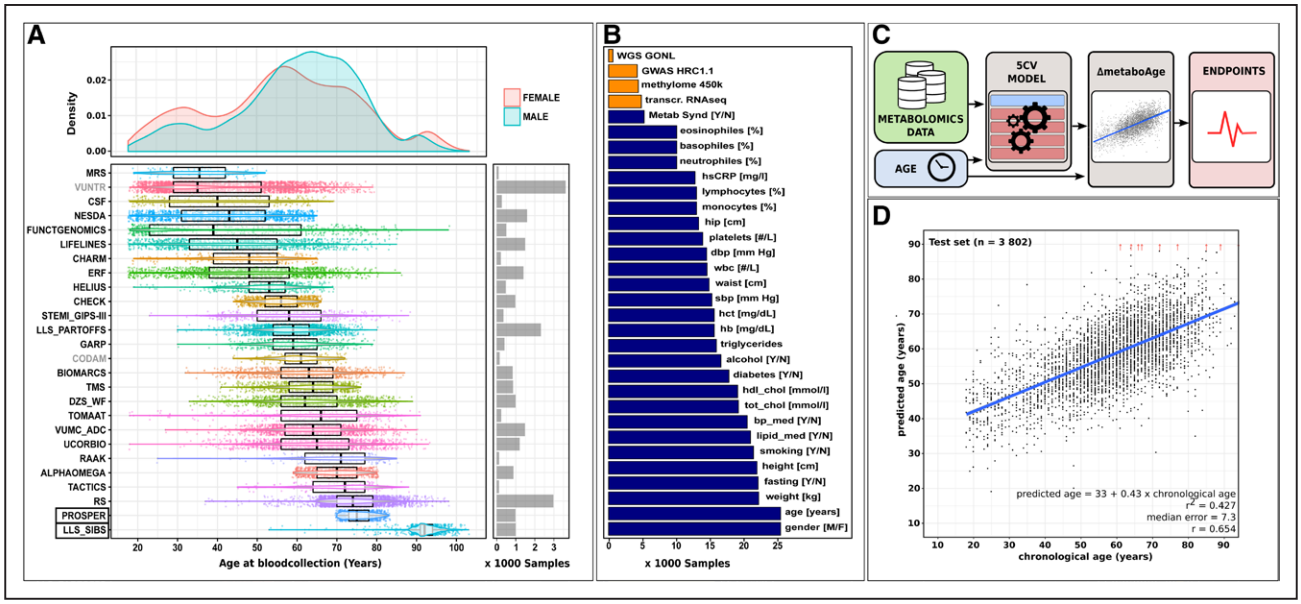

Figure 1. Biobanking and Biomolecular Resources Research Infrastructure the Netherlands (BBMRI-NL) is a vast proton nuclear magnetic resonance (^{1}H-NMR) metabolomics resource enabling approaches for personalized medicine.
A, Cohorts in the Dutch Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL), totaling 25 253 samples display interlinked age distributions robustly covering the complete adult life span from 18 till 85 y. While, VUNTR (Vrije Universiteit Netherlands Twin Register) and CODAM (Cohort on Diabetes and Atherosclerosis Maastricht; gray) were omitted for training the age predictor due to incomplete data (Methods), LLS_SIBS and PROSPER (boxed) were held out to independently evaluate the merit of age predictions as surrogate biomarkers for clinical end points. **B**, Additional omics data (orange) and phenotypic variables (blue) available within the BBMRI-NL resource. **C**, Flowchart of the analyses: a predictor for chronological age is trained on BBMRI-NL metabolomics data. The age-independent part of differences between predicted age and chronological age, termed ΔmetaboAge, is associated with end points. **D**, Five-Fold-Cross Validation (5CV) is performed to assess the accuracy of the age predictor. Predictions on the test set of a representative fold are depicted, with ΔmetaboAge exemplified in orange. F indicates female; M, male; N, no; and Y, yes.

using phenotypic data available within the BBMRI-NL resource (see Document I in the Data Supplement for distribution and availability of phenotypic data per cohort). Meta-analyses across biobanks showed that a positive ΔmetaboAge corresponded with a poor cardiometabolic health, as represented by higher body mass index (BMI), higher serum levels of C-reactive protein, and not unsurprisingly, higher cholesterol and triglycerides. In addition, use of blood pressure–lowering medication, but not lipid-lowering medication, is associated with a higher ΔmetaboAge (Figure 2A; Document I in the Data Supplement for results per cohort). These associations remained significant when further adjusted for sex and BMI (Table VI in the Data Supplement).

Associations of metaboAge With Current and Future Cardiometabolic Disease

Next, we investigated whether ΔmetaboAge marks current and future clinical metabolic disease end points. Participants with current metabolic syndrome or diabetes mellitus type 2 were consistently estimated older as compared with their healthy counterparts of similar age (Figure 2B), with diabetes mellitus type 2 remaining significant when also adjusting for sex and BMI (Table VII in the Data Supplement). The predictive value of ΔmetaboAge for future cognitive and cardiometabolic disease was tested in the PROSPER study,[17] a multicenter clinical trial investigating the efficacy of lipid-lowering medication for elderly patients (70–82 years) at risk of cardiovascular events followed for a median follow-up time of 3.3 years (Table II in the Data Supplement). While at most marginal correlations were observed between ΔmetaboAge and measures of cognitive decline at baseline (Table VIII in the Data Supplement) or during follow-up (Table IX in the Data Supplement), patients with a positive ΔmetaboAge were shown to be at risk of future coronary and cardiovascular events independent of sex, BMI, smoking status, diabetes mellitus type 2 status, antihypertensive medication, and pravastatin treatment (Figure 2C). Using the same model, we found patients with a positive ΔmetaboAge to be at increased risk of heart failure hospitalization and vascular and all-cause mortality (Figure 2C).

Associations of metaboAge With Mortality and Functionality in the Oldest Old

Finally, we evaluated whether ΔmetaboAge marks biological aging near the extremes of human life span. We examined participants of the LLS_SIBS,[18] aged ≥89 years and followed during a median follow-up time of

A Associations of cardio-metabolic risk factors with ΔmetaboAge in BBMRI-NL

Risk Factor	β	95% CI	*p*-value	N
Gender [M/F]	-0.35	-0.84 – -0.15	0.174	18,227
Body Mass Index [kg m^{-2}]	0.24	0.20 – 0.28	**2.59 x 10^{-33}**	16,132
smoking [Y/N]	0.25	-0.04 – 0.54	0.094	15,520
Total Cholesterol [mmol l^{-1}]	0.82	0.59 – 1.05	**2.76 x 10^{-12}**	14,807
HDL Cholesterol [mmol l^{-1}]	-1.08	-1.76 – 0.40	**1.94 x 10^{-03}**	14,722
lipid lowering med. [Y/N]	0.65	-0.07 – 1.37	0.0754	13,329
Systolic Blood Pressure [mm Hg]	0.01	-0.01 – 0.02	0.48	12,895
Diastolic Blood Pressure [mm Hg]	0.01	-0.02 – 0.04	0.44	12,387
Bloodpressure lowering med [Y/N]	1.70	1.21 – 2.19	**8.98 x 10^{-12}**	12,579
Triglycerides* [mmol l^{-1}]	2.20	1.30 – 3.11	**1.64 x 10^{-06}**	11,526
alcohol usage [Y/N]	-0.73	-1.05 – -0.41	**5.61 x 10^{-06}**	11,425
hsCRP* [mg l^{-1}]	1.00	0.62 – 1.37	**1.76 x 10^{-07}**	7,567

B Associations of prevalent cardio-metabolic disease with ΔmetaboAge in BBMRI-NL

Phenotype	OR	95% CI	*p*-value	N_{case}	$N_{control}$
Metabolic Syndrome [Y/N]	2.77	1.54 – 3.99	**9.36 x 10^{-06}**	1,635	1,950
Type 2 Diabetes [Y/N]	2.52	1.93 – 3.11	**5.10 x 10^{-17}**	1,381	11,252

C Associations of incident cardio-metabolic disease with ΔmetaboAge in PROSPER

Phenotype	HR	95% CI	*p*-value	N_{case}	$N_{control}$
Coronary events	1.25	1.11 – 1.40	**2.64 x 10^{-04}**	569	4,851
Cardiovascular events	1.20	1.08 – 1.33	**4.86 x 10^{-04}**	865	4,555
Vascular mortality	1.57	1.31 – 1.88	**8.56 x 10^{-07}**	237	5,183
All-cause mortality	1.42	1.25 – 1.61	**9.14 x 10^{-08}**	477	4,943
Heart failure hospitalisation	1.68	1.37 – 2.06	**5.42 x 10^{-07}**	189	5,324

D Association of mortality with ΔmetaboAge in LLS_SIBS

HR	95% CI	*p*-value	N_{event}	N_{total}
1.25	1.14 – 1.37	**2.43 x 10^{-06}**	793	811

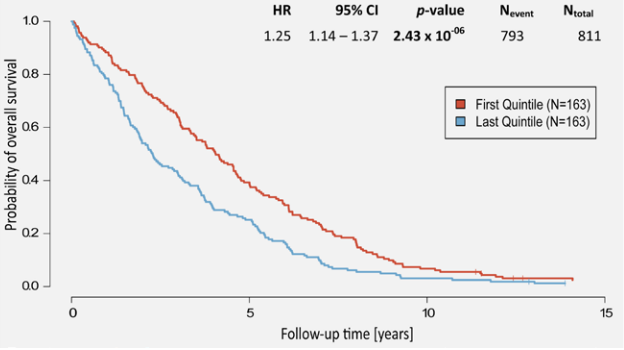

Figure 2. Associations of ΔmetaboAge with (risk factors of) cardiometabolic disease risk and all-cause mortality. Associations with (**A**) association of cardiometabolic risk factors with ΔmetaboAge in Biobanking and Biomolecular Resources Research Infrastructure the Netherlands (BBMRI-NL). **B**, Association of prevalent cardiometabolic disease with ΔmetaboAge in BBMRI-NL. **C**, Association of incident cardiometabolic disease with ΔmetaboAge in PROSPER. **D**, Association of mortality with ΔmetaboAge in LLS_SIBS adjusted for age and sex. A Kaplan-Meijer curve illustrates the difference in mortality between quintiles with the highest (blue; estimated ≥6.9 y older) and the lowest (red; estimated ≥7.3 y younger) ΔmetaboAge. βs are reported as increase in ΔmetaboAge per unit of increase in the risk factor (**A**) or disease status (**B**). Hazard ratios (HRs) reported as increased risk per 10-y of ΔmetaboAge. *P* values are in bold when significant after correction for multiple testing (Bonferroni). F indicates female; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; M, male; med, medication; N, no; OR, odds ratio; and Y, yes. *Log-transformed.

12.4 years for all-cause mortality (Table II in the Data Supplement). At baseline, a positive ΔmetaboAge correlated with lower instrumental activities of daily living (P=2.0×10^{-16})—a measure of physical independence. Moreover, a positive ΔmetaboAge also marked nonagenarians at an increased risk of all-cause mortality (Figure 2D) during 10 years of follow-up, even when adjusting for instrumental activities of daily living (Table X in the Data Supplement).

DISCUSSION

We present a rich resource of ^{1}H-NMR serum metabolomics and routine serum measurements encompassing over 25 000 samples, derived from 26 community- and hospital-based cohorts (download data access request at bbmri.nl/samples-images-data). Using this resource, we have constructed a score reflecting an individual's biological age, called metaboAge, and demonstrate that the excess of metaboAge over chronological age (ΔmetaboAge) confers an increased risk for future cardiovascular disease, mortality up to the highest ages, and functionality among older adults. Lastly, we have made a web-based tool available at metaboage.researchlumc.nl facilitating an easy incorporation of ΔmetaboAge scores in future epidemiological studies.

We evaluated the applicability of ΔmetaboAge as a biomarker for current and future cardiometabolic health and disease as the same metabolomics platform has previously been successfully used to predict outcomes for cardiovascular disease[16] and type 2 diabetes.[19] In line with these papers, we observed that higher ΔmetaboAge indicates various aspects of current and future cardiometabolic health, including significant associations with BMI (P=2.59×10^{-33}), C-reactive protein (P=1.76×10^{-07}), current type 2 diabetes mellitus (P=5.10×10^{-17}), future cardiovascular events (P=2.64×10^{-04}), and vascular mortality (P=8.56×10^{-07}). Hence, ΔmetaboAge can be readily explored, also in studies lacking cardiometabolic risk factors or end points, as a surrogate marker to capture some aspects of current or future cardiometabolic health.

Ideally, biomarkers of biological age are broadly applicable and are thus indicative of one or several of the 5 health domains as defined by Lara et al.[20] Whereas we showed that ΔmetaboAge is indicative of classical biomarkers belonging to the physiological (cardiovascular health), immune (high-sensitivity C-reactive protein), and physical capability domain (Instrumental Activities

of Daily Living), we were unable to establish significant correlations with classical biomarkers of the cognitive or endocrine domain. This was either because we lacked the classical biomarkers, as for the endocrine domain, or that ΔmetaboAge did not correlate with the available classical biomarkers, as for the cognitive domain. Of note is that a measure not available to us, general cognitive ability, has recently been reported to associate with several metabolite measurements of this platform in a large epidemiological study.[21] Hence, we expect that future large-scale metabolomics studies using the Nightingale platform, for example, the UK Biobank, will shed more light on other aspects of biological age indicated by ΔmetaboAge.

We have used 2 evaluation procedures to get an unbiased estimate of the model performance of our ^{1}H-NMR metabolomics-based predictor for chronological age under 2 different though complementary scenarios. First, we have used 5CV splitting the data into 5 training and test sets in which all train and tests sets have similar age and sex distributions. As this method takes samples from all evaluated biobanks, it intrinsically conditions on potential batch effects and can, therefore, be too optimistic. To specifically evaluate the scenario of unseen biobanks, we also performed a LOBOV. While this method more realistically captures variation introduced between biobanks, it suffers, due to the choice of the Pearson correlation between predicted and chronological age as an evaluation measure, from the considerable differences in sample sizes and age ranges between biobanks. Hence results with LOBOV might be overly conservative. Collectively, the 5CV and LOBOV results should provide sensible estimate on the performance of the proposed metabolomics-based age predictor.

While the blood metabolome can be readily assessed using ^{1}H-NMR metabolomics at high throughput, high reproducibility, and low costs, no ^{1}H-NMR metabolomics clock has to date been made available. We have applied the clock paradigm popularized by the work of Horvath et al[8] to derive such a metabolomics-based predictor of age for a metabolomics platform commonly used in large epidemiological studies. Similarly, we have shown that our clock associates with various clinical end points including mortality. While clock algorithms have become increasingly popular as a means to perform sample stratification, an important limitation of the clock paradigm remains that it is hard to trace back why such scores reflect aspects of current and future disease, let alone for which disease applications a particular score is most suitable. Hence, newly proposed scores inevitably require additional empirical evidence in other epidemiological cohorts to support its added value. To accommodate future research with ΔmetaboAge, we have made a web-based tool available at metaboage.researchlumc.nl. Lastly, ongoing research on clock algorithms also generates new knowledge on the methodology how such health predictors could be derived. Here we made the conservative decision to omit metabolites measured with low success rates (<98%) or that frequently failed to reach the detection limit (<98%), thus potentially ignoring the fact that these aspects might be informative on aging processes. Hence, to also accommodate future research into newly created clocks or other scores, data access can be requested at bbmri.nl/samples-images-data.

In summary, we present a rich resource of ^{1}H-NMR serum metabolomics and routine serum measurements encompassing over 25 000 samples (download data access request at bbmri.nl/samples-images-data). Moreover, we illustrate the merit of such a resource by presenting ΔmetaboAge—a novel metabolomics-based indicator of biological age capturing aspects of current and future cardiometabolic health (predictions available at metaboage.researchlumc.nl).

ARTICLE INFORMATION

Received May 14, 2019; accepted July 27, 2020.

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Sources of Funding

Financial support for the Alpha Omega Cohort was obtained from the Dutch Heart Foundation (grant 200T401) and the National Institutes of Health (grant R01HL076200). DNA isolation was funded by Biobanking and Biomolecular Resources Research Infrastructure the Netherlands (BBMRI-NL; grant CP2011-18). Amsterdam Dementia Cohort: research within the VUmc Alzheimer Center is part of the neurodegeneration research program of Amsterdam Neuroscience supported by Alzheimer Nederland and Stichting VUmc fonds. LUMINA is supported by grants obtained from the Netherlands Organization for the Health Research and Development (ZonMw; No. 90700217) and VIDI (ZonMw; No. 91711319); the Netherlands Organisation for Scientific Research (NWO) VICI (No. 918.56.602) and Spinoza Prize (2009) grants; the Centre for Medical Systems Biology (CMSB) and Netherlands Consortium for Systems Biology (NCSB), both within the framework of the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO), the Seventh Framework EU Project EUROHEADPAIN (No. 602633). CHECK was funded by the Dutch Arthritis Association. CODAM: part of this work was supported by grants of NWO (940-35-034) and the Dutch Diabetes Research Foundation (98.901). TMS (The Maastricht Study) was supported by the European Regional Development Fund via OP-Zuid, the Province of Limburg, the Dutch Ministry of Economic Affairs (grant 310.041), Stichting De Weijerhorst (Maastricht, the Netherlands), the Pearl String Initiative Diabetes (Amsterdam, the Netherlands), CARIM School for Cardiovascular Diseases (Maastricht, the Netherlands), Stichting Annadal (Maastricht, the Netherlands), Health Foundation Limburg (Maastricht, the Netherlands), and by unrestricted grants from Janssen-Cilag B.V. (Tilburg, the Netherlands), Novo Nordisk Farma B.V. (Alphen aan den Rijn, the Netherlands), and Sanofi-Aventis Netherlands B.V. (Gouda, the Netherlands). The DCS study (Hoorn Diabetes Care System cohort) was been made possible by collaboration with the Diabetes Care System West-Friesland. The ERF study (Erasmus Rucphen Family) has received funding from the CMSB and NCSB, both within the framework of NGI/Netherlands Organization for Scientific Research (NWO). The ERF study is also a part of the European Special Populations Research Network (FP6 STREP grant number 18947 [LSHG-CT-2006-018947]); European Network of Genomic and Genetic Epidemiology (ENGAGE) from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413; "Quality of Life and Management of the Living Resources" of Fifth Framework Programme (No. QLG2-CT-2002-01254); FP-7 project EUROHEADPAIN (nr. 602633), the Internationale Stichting Alzheimer Onderzoek; the Hersenstichting Nederland; and the JNPD under the project PERADES (grant number 733051021, Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease using multiple powerful cohorts, focused Epigenetics and Stem cell metabolomics). Metabolomics Measurements of ERF has been funded by BBMRI-NL (184.021.007). The ERF follow-up study is funded by CardioVasculair Onderzoek Nederland (CVON 2012-03). Rotterdam Study is supported by the Erasmus MC University Medical Center and Erasmus University Rotterdam; NWO; the Netherlands Organisation for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly; NGI; the Ministry of Education, Culture and Science; the Ministry of Health, Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam. Metabolomics measurements were funded by BBMRI-NL (184.021.007) and the JNPD under the project PERADES (grant number 733051021, Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease using multiple powerful cohorts, focused Epigenetics and Stem cell metabolomics). GARP: the Leiden University Medical Centre has and is supporting the RAAK and GARP studies. This study was supported by the Dutch Arthritis Foundation and Pfizer Groton, Connecticut. We are indebted to Drs N. Riyazi, J. Bijsterbosch, H.M. Kroon, and I. Watt for collection of data. The HELIUS study is conducted by the Academic Medical Center Amsterdam and the Public Health Service of Amsterdam. Both organizations provided core support for HELIUS. The HELIUS study is also funded by the Dutch Heart Foundation, the Netherlands Organization for Health Research and Development (ZonMw), the European Union (FP-7), and the European Fund for the Integration of non-EU immigrants (EIF).The LLS (Leiden Longevity Study) has received funding from the European Union Seventh Framework Programme (FP7/2007-2011) under grant agreement No. 259679. This study was supported by a grant from the Innovation-Oriented Research Program on Genomics (SenterNovem IGE05007), CMSB, and the Netherlands Consortium for Healthy Ageing (grants 05040202 and 050-060-810), all in the framework of NGI, Netherlands Organization for Scientific Research (NWO), Unilever Colworth, and by BBMRI-NL, a research infrastructure financed by the Dutch government (NWO 184.021.007). The infrastructure for the NESDA study (www.nesda.nl) is funded through the Geestkracht program of ZonMw (grant number 10-000-1002) and financial contributions by participating universities and mental healthcare organizations (VU University Medical Center, GGZ inGeest, Leiden University Medical Center, Leiden University, GGZ Rivierduinen, University Medical Center Groningen, University of Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Rob Giel Onderzoekscentrum). PROSPER was supported by an investigator-initiated grant obtained from Bristol-Myers Squibb. Prof Dr J.W. Jukema is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). PROSPER was supported by the European Federation of Pharmaceutical Industries Associations, Innovative Medicines Initiative Joint undertaking, European Medical Information Framework grant number 115372, and the European Commission under the Health Cooperation Work Programme of the Seventh Framework Programme (grant number 305507) "Heart 'omics' in Ageing". The LUMC arthroplasty studies are a combination of TACTICS, TOMAAT, and RAAK cohorts. TACTICS was funded by the Dutch Board of Health Care Insurances (College voor Zorgverzekeringen; OG99/023) and Sanquin Blood Bank. Funding for the TOMAAT study was received from ZonMW (06-601) and Sanquin Blood Supply (03-002), the Netherlands. Unique identifier: ISRCTN96327523 (controlled-trials.com) and NTR 303 (Dutch Trial Register). The RAAK study was supported by the Leiden University Medical Centre. Furthermore, the molecular studies performed within the RAAK study have received funding from the Dutch Arthritis Association (DAA_10_1-402), BBMRI-NL complementation project CP2013-84-CP2013-83, and Dutch Scientific Research Council NWO/ZonMW VICI scheme (nr. 91816631/528). UCORBIO is conducted and supported by the Department of Cardiology, University Medical Center Utrecht, the Netherlands. Dr Asselbergs is supported by UCL Hospitals NIHR Biomedical Research Centre. Metabolic profiling was supported by BBMRI-NL. UCORBIO received funding from FP EU project CVgenes@target (HEALTH-F2-2013–601456). VUNTR: funding was obtained from the Netherlands Organization for Scientific Research (NWO) and MagW/ZonMW grants 904-61-090, 985-10-002, 904-61-193, 480-04-004, 400-05-717, Addiction-31160008, Middelgroot-911-09-032, Spinozapremie 56-464-14192, and BBMRI-NL (184.021.007); the European Community Seventh Framework Program (FP7/2007-2013), ENGAGE (HEALTH-F4-2007-201413); the European Science Council (ERC Advanced, 230374), Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota, and the National Institutes of Health (R01D0042157-01A, MH081802, Grand Opportunity grant 1RC2 MH089951). We gratefully acknowledge grant NWO 480-15-001/674: Netherlands Twin Registry Repository: researching the interplay between genome and environment.

Disclosures

None.

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