Dairy consumption, systolic blood pressure, and risk of hypertension

Mendelian randomization study

Ding, Ming; Huang, Tao; Bergholdt, Helle K M; Sørensen, Thorkild I.A.; Linneberg, Allan René; Sandholt, Camilla Helene; Pedersen, Oluf ; Hansen, Torben; Kilpeläinen, Tuomas O; CHARGE Consortium; Nordestgaard, Børge G; Ellervik, Christina; Qi, Lu; Mendelian Randomization of Dairy Consumption Working Group

Published in:
The BMJ

DOI:
10.1136/bmj.j1000

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC

Citation for published version (APA):
Dairy consumption, systolic blood pressure, and risk of hypertension: Mendelian randomization study

Mendelian Randomization of Dairy Consumption Working Group

**ABSTRACT**

**OBJECTIVE**

To examine whether previous observed inverse associations of dairy intake with systolic blood pressure and risk of hypertension were causal.

**DESIGN**

Mendelian randomization study using the single nucleotide polymorphism rs4988235 related to lactase persistence as an instrumental variable.

**SETTING**

CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium.

**PARTICIPANTS**

Data from 22 studies with 171 213 participants, and an additional 10 published prospective studies with 26 119 participants included in the observational analysis.

**MAIN OUTCOME MEASURES**

The instrumental variable estimation was conducted using the ratio of coefficients approach. Using meta-analysis, an additional eight published randomized clinical trials on the association of dairy consumption with systolic blood pressure were summarized.

**RESULTS**

Compared with the CC genotype (CC is associated with complete lactase deficiency), the CT/TT genotype (TT is associated with lactose persistence, and CT is associated with certain lactase deficiency) of LCT-13910 (lactase persistence gene) rs4988235 was associated with higher dairy consumption (0.23 (about 55 g/day), 95% confidence interval 0.17 to 0.29 serving/day; P<0.001) and was not associated with systolic blood pressure (0.31, 95% confidence interval −0.05 to 0.68 mm Hg; P=0.09) or risk of hypertension (odds ratio 1.01, 95% confidence interval 0.97 to 1.05; P=0.27). Using LCT-13910 rs4988235 as the instrumental variable, genetically determined dairy consumption was not associated with systolic blood pressure (β=1.35, 95% confidence interval −0.28 to 2.97 mm Hg for each serving/day) or risk of hypertension (odds ratio 1.04, 0.88 to 1.24). Moreover, meta-analysis of the published clinical trials showed that higher dairy intake has no significant effect on change in systolic blood pressure for interventions over one month to 12 months (intervention compared with control groups: β=−0.21, 95% confidence interval −0.98 to 0.57 mm Hg). In observational analysis, each serving/day increase in dairy consumption was associated with −0.11 (95% confidence interval −0.20 to −0.02 mm Hg; P=0.02) lower systolic blood pressure but not risk of hypertension (odds ratio 0.98, 0.97 to 1.00; P=0.11).

**CONCLUSION**

The weak inverse association between dairy intake and systolic blood pressure in observational studies was not supported by a comprehensive instrumental variable analysis and systematic review of existing clinical trials.

**WHAT IS ALREADY KNOWN ON THIS TOPIC**

Observational studies showed that dairy intake was associated with lower systolic blood pressure and lower risk of hypertension

**WHAT THIS STUDY ADDS**

Using a Mendelian randomization approach, we found that genetically determined dairy consumption was not associated with systolic blood pressure or risk of hypertension.
risk of hypertension. In addition we conducted a meta-analysis to summarize the results of eight randomized clinical trials assessing dairy intake intervention on changes in systolic blood pressure.

Methods
Study design and population
We used an instrumental variable approach to examine associations of dairy consumption with systolic blood pressure and risk of hypertension. We collected data from 22 observational studies with 171,213 participants within the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium. All participants provided written informed consent. The web appendix describes the studies in the analysis.

To provide comprehensive evidence on associations of dairy intake with systolic blood pressure and risk of hypertension, we conducted a systematic review of previously published cohort studies and randomized clinical trials. In the web appendix, we describe the process of the systematic review in detail.

Dairy consumption
Dairy products included skim/low fat milk, whole milk, ice cream, yogurt, cottage/ricotta cheese, cream cheese, other cheese, and cream. In most of the studies, dairy intake was self reported by food frequency questionnaire. We calculated total dairy consumption as the sum of all dairy categories (see table 1 in the web appendix for a detailed description of dairy consumption in the included studies).

Outcome measures
The outcome of our Mendelian randomization included systolic blood pressure and risk of hypertension. Given that systolic blood pressure is superior to diastolic blood pressure as a major risk factor of cardiovascular disease, we used systolic blood pressure as the main outcome in our analysis (see table 1 in the web appendix for the detailed measurement of systolic blood pressure in the included studies). For participants taking antihypertensive drugs, we added 15 mm Hg to systolic blood pressure to adjust for treatment effects. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher or current use of antihypertensive drugs.

SNP rs4988235
Table 1 in the web appendix shows genotyping platforms, genotype frequencies, Hardy-Weinberg equilibrium P values, and call rates for lactase persistence SNP rs4988235. The SNP rs4988235 was not genotyped or imputed in two studies; proxy SNPs (rs309137: $r^2=0.77$; rs1446585: $r^2=1.00$) were used instead.

Statistical analyses
We initially conducted statistical analyses within each included study in accordance with a standard analysis plan. As lactase persistence is inherited as a dominant trait, we used dominant models (CC vs CT/TT genotype) to examine associations of LCT-13910 rs4988235 with dairy intake, systolic blood pressure, and risk of...
hypertension adjusting for baseline age, sex, ethnicity, and region. We examined associations of dairy consumption with systolic blood pressure and risk of hypertension using linear or logistic models adjusting for baseline age, body mass index, blood pressure, smoking status, physical activity, total energy intake, and alcohol consumption at baseline.

We included 22 studies with 171,213 participants from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium. Table 1 shows the baseline characteristics of the studies. Of the 22 studies, nine were conducted in the US, nine in countries in northern Europe, three in countries in southern Europe, and one in Australia. The frequency of CC alleles varied across studies. In most of the studies, participants were estimated to have a systolic blood pressure of 130 mm Hg or higher and a prevalence of hypertension of at least 20%.

We also conducted stratified analyses on the causal estimates of dairy intake with systolic blood pressure and risk of hypertension by frequency of CC alleles (≤12%, >12%), region or country (northern Europe, southern Europe, US), race (white, other), study design (cross-sectional, prospective), and measurement of systolic blood pressure (self-reported, clinical). We used meta-regression to evaluate effect modification by each study level characteristics. In sensitivity analyses, we applied instrumental variable analysis within each study and combined the instrumental variable estimates through meta-analysis; we repeated our analyses using additive (we assumed 0, 1, and 2 for TT, CT, and CC alleles) and recessive models (CC/CT v TT). We conducted restriction analyses by excluding studies that used proxy SNPs, studies that used LCT-13910 rs4988235 in Hardy-Weinberg disequilibrium, or studies where LCT-13910 rs4988235 was not statistically significantly associated with higher dairy intake.

All meta-analyses were conducted at Harvard TH Chan School of Public Health using Stata version 11.2 (STATA Corp, College Station, TX).

Patient involvement
No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for design or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community.

Results
We included 22 studies with 171,213 participants from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium. Table 1 shows the baseline characteristics of the studies. Of the 22 studies, nine were conducted in the US, nine in countries in northern Europe, three in countries in southern Europe, and one in Australia. The frequency of CC alleles varied across studies. In most of the studies, participants were estimated to have a systolic blood pressure of 130 mm Hg or higher and a prevalence of hypertension of at least 20%.

We further conducted stratified analysis on the causal estimates of dairy intake with systolic blood pressure and risk of hypertension by frequency of CC alleles (≤12%, >12%), region or country (northern Europe, southern Europe, US), race (white, other), study design (cross-sectional, prospective), and measurement of systolic blood pressure (self-reported, clinical). We used meta-regression to evaluate effect modification by each study level characteristics. In sensitivity analyses, we applied instrumental variable analysis within each study and combined the instrumental variable estimates through meta-analysis; we repeated our analyses using additive (we assumed 0, 1, and 2 for TT, CT, and CC alleles) and recessive models (CC/CT v TT). We conducted restriction analyses by excluding studies that used proxy SNPs, studies that used LCT-13910 rs4988235 in Hardy-Weinberg disequilibrium, or studies where LCT-13910 rs4988235 was not statistically significantly associated with higher dairy intake.

All meta-analyses were conducted at Harvard TH Chan School of Public Health using Stata version 11.2 (STATA Corp, College Station, TX).
white, and dairy intake was assessed prospectively before measuring systolic blood pressure.

By conducting a systematic review, we additionally identified 10 published cohort studies with 26,119 participants and eight randomized clinical trials with 735 participants. Figure 1 in the web appendix shows the flowchart of study selection. The clinical trials examined the effect of dairy intake on systolic blood pressure over one month to 12 months of interventions. In the cohort studies, seven assessed systolic blood pressure as the outcome and five used hypertension as the outcome. Tables 2 and 3 in the web appendix show the characteristics of the published trials and cohorts.

In observational analysis, each serving/day increase in dairy consumption was associated with lower systolic blood pressure (β=−0.11, 95% confidence interval −0.20 to −0.02 mm Hg; P=0.02) and was not associated with a lower relative risk of hypertension (odds ratio 0.98, 95% confidence interval 0.97 to 1.00; P=0.11) (figs 1 and 2). In the randomized clinical trials, however, dairy intake did not show a significant effect on changes in systolic blood pressure over one month to 12 months of interventions (comparing intervention with control group: β=−0.21, −0.98 to 0.57 mm Hg; P=0.60) (fig 3). No publication bias of included cohorts and clinical trials was found (systolic blood pressure in cohorts: Egger’s test P=0.51; hypertension in cohorts: P=0.46; randomized clinical trials: P=0.33) (fig 2 in the web appendix).

Compared with the CC genotype, the CT/TT genotype of LCT-13910 rs4988235 was associated with higher dairy consumption (0.23 (95% confidence interval 0.17 to 0.29) serving/day (about 55 g/day); P<0.001), and the Z statistic was 7.51, showing that the instrumental variable was strong and valid (fig 4). However, significant heterogeneity was found across studies (I²=80.0%; P<0.001 for heterogeneity). Compared with the CC genotype, the CT/TT genotype of LCT-13910 rs4988235 was not associated with systolic blood pressure (0.31, −0.05 to 0.68 mm Hg; P=0.09) or risk of hypertension (odds ratio 1.01, 95% confidence interval 0.97 to 1.05; P=0.27) (figs 5 and 6). Using LCT-13910 rs4988235 as the instrumental variable, we estimated that genetically determined dairy consumption was not associated with systolic blood pressure (β=1.35, 95% confidence interval −0.28 to 2.97 mm Hg for each serving/day) or risk of hypertension (odds ratio 1.04, 0.88 to 1.24).

To explore sources of heterogeneity in the association of LCT-13910 rs4988235 with dairy intake, we conducted stratified analyses by region or country, frequency of the CC genotype, race, study design, and measurement of systolic blood pressure. We classified Denmark, the Netherlands, Sweden, and Finland as northern European countries and Italy, Spain, and France as southern European countries. Among studies with a CC genotype frequency of 12% or less, or studies conducted in northern European countries, we found no heterogeneity of LCT-13910 rs4988235 with dairy intake, and the instrumental variable remained strong in both subgroups. Genetically determined dairy consumption was unrelated to systolic blood pressure and risk of hypertension within each stratum, which was consistent with the main finding.
In this study, using Mendelian randomization analysis in 32 studies (22 observational studies, 10 previously published cohort studies) with 197,332 participants, we examined the potential causal effect of dairy consumption on systolic blood pressure and risk of hypertension. Using the \textit{LCT-13910} gene variant affecting lactase persistence as the instrumental variable, our study showed that genetically determined dairy intake did not affect systolic blood pressure or risk of hypertension. Furthermore, a meta-analysis of the results from published randomized clinical trials showed that dairy consumption had no effect on changes of systolic blood pressure in response to interventions over one month to 12 months.

**Discussion**

In this study, using Mendelian randomization analysis in 32 studies (22 observational studies, 10 previously published cohort studies) with 197,332 participants, we examined the potential causal effect of dairy consumption on systolic blood pressure and risk of hypertension. Using the \textit{LCT-13910} gene variant affecting lactase persistence as the instrumental variable, our study showed that genetically determined dairy intake did not affect systolic blood pressure or risk of hypertension. Furthermore, a meta-analysis of the results from published randomized clinical trials showed that dairy consumption had no effect on changes of systolic blood pressure in response to interventions over one month to 12 months.

**Strengths and weaknesses of this study**

Our study has several strengths. First, we carried out a large instrumental variable analysis on the causality of dairy intake on systolic blood pressure and hypertension. The large sample size provided us with enough power to estimate the causal effect of dairy intake on systolic blood pressure. Second, the single nucleotide polymorphism (SNP) \textit{rs4988235} for lactase persistence is a well established variant associated with dairy intake, with a solid biological basis, and is therefore a highly valid instrumental variable. Third, we summarized published randomized clinical trials on dairy consumption with systolic blood pressure. Although clinical trials have shorter follow-up time than cohort studies, they still provided further supportive evidence to the instrumental variable results.

Our study has several limitations. First, given the variability of the CC allele across studies and the different prevalence of hypertension across countries, population stratification might exist. However, as most of the studies included were genetically homogeneous, we performed instrumental variable analysis within each study first and...
combined the instrumental variable results through meta-analysis. The instrumental variable results were consistent with the main findings. Second, the pleiotropic effect of SNP rs4988235 is not known. However, SNP rs4988235 was located in the MCM6 gene upstream from LCT-13910, and neither gene has been found to have additional biological function besides lactase persistence.13 Third, dairy consumption was self-reported by questionnaire and might be affected by measurement errors. If measurement errors were random, the observed associations would be biased to the null. However, the results for instrumental variable estimates would not be biased, although the confidence interval might be larger. Fourth, we included total dairy intake as the main exposure; however, lactase content differs between dairy products. For example, Swiss cheese and mozzarella contain trivial amounts of lactase. Similar to the measurement error of dairy intake, the variability in lactase content of dairy products might not bias the instrumental variable estimates but might widen the confidence intervals. Fifth, several studies examined dairy consumption and systolic blood pressure using a cross-sectional study design, and even if instrumental variable analysis was used this might result in reverse causation. However, no statistically significant effect modification by study design was found in stratified analysis, indicating that reverse causation caused by study design might be minimal.

Fig 6 | Association of SNP rs4988235 with relative risk of hypertension using dominant model (CT/TT vs CC genotype). Logistic regression adjusted for baseline age, sex, ethnicity, and region or country.

Strengths and weaknesses in relation to other studies

In our study we observed an inverse association between dairy intake and systolic blood pressure. Consistently, cross-sectional studies showed an inverse association between dairy intake and systolic blood pressure. Previous cohort studies have been summarized in two meta-analyses. One meta-analysis involving approximately 45,000 participants showed that dairy products were associated with lower risks of raised systolic blood pressure. In line with this, another meta-analysis, which included nine cohort studies with a sample size of 57,256, found an inverse association between dairy foods and risk of hypertension. However, in both meta-analyses, the associations of high fat dairy products, including whole milk, cream, and cream cheese, and low fat dairy products, including skim milk and yogurt with systolic blood pressure were inconsistent. In the two published meta-analyses, the observed inverse association was mainly due to consumption of low fat dairy products. Furthermore, a meta-analysis summarizing 14 clinical trials found that probiotic fermented milk, including yogurt, resulted in a statistically significant reduction in systolic blood pressure. Clinical trials also showed that tripeptides and peptides derived from milk have hypotensive effects in prehypertensive and hypertensive participants.

Possible explanations and implications

Compared with the CC genotype, the CT/TT genotype was associated with 0.23 serving/day (about 55 g/day) higher dairy intake. In previous cohort studies, a 55 g/day increment in dairy intake was estimated to be statistically significantly associated with 0.03 mm Hg lower systolic blood pressure, and 1%, 2%, and 1% lower risks of hypertension, type 2 diabetes, and cardiovascular disease, respectively. However, in our study, the CT/TT genotype was associated with a 0.31 mm Hg higher systolic blood pressure, and genetically determined dairy consumption did not decrease systolic blood pressure using instrumental variable estimation. Moreover, the meta-analyzed results of clinical trials showed that dairy intake had no effect on changes in systolic blood pressure. There could be two reasons that the reported associations from observational studies were inconsistent with our instrumental variable results. First, even if yogurt and specific nutrients in dairy such as milk peptides have antihypertensive effects, specific dairy products such as yogurt only compose a small fraction of total dairy products and could not explain the general observational association between dairy intake and outcome. Second, higher low fat dairy intake was more likely to be associated with a healthy diet and lifestyle. Therefore, the observed inverse association of particularly low fat dairy intake with systolic blood pressure might be due to confounding of intake of other food items and a healthy lifestyle. However, as one fundamental assumption for the instrumental variable to be valid is that the instrumental variable is associated with the outcome only through the exposure under study.
**Table 2:** Stratified analysis on causal estimates of dairy consumption (serving/day) with systolic blood pressure (mm Hg) and risk of hypertension (odds ratio). Values in brackets are 95% confidence intervals unless stated otherwise.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SNP rs4988235 (F allele frequency)</th>
<th>SNP rs4988235 (Z statistic)</th>
<th>SNP rs4988235 (P value)</th>
<th>SNP rs4988235 (F allele frequency)</th>
<th>SNP rs4988235 (Z statistic)</th>
<th>SNP rs4988235 (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>20</td>
<td>0.23 (0.03 to 0.43)</td>
<td>27.3 (0.000)</td>
<td>0.016 (0.000)</td>
<td>0.016 (0.000)</td>
<td>0.016 (0.000)</td>
</tr>
<tr>
<td>African</td>
<td>4</td>
<td>0.20 (0.00 to 0.41)</td>
<td>29.5 (0.000)</td>
<td>0.016 (0.000)</td>
<td>0.016 (0.000)</td>
<td>0.016 (0.000)</td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
<td>0.26 (0.16 to 0.38)</td>
<td>4.71 (0.030)</td>
<td>0.16 (0.000)</td>
<td>0.16 (0.000)</td>
<td>0.16 (0.000)</td>
</tr>
<tr>
<td>Age</td>
<td>5</td>
<td>0.21 (0.01 to 0.42)</td>
<td>2.01 (0.044)</td>
<td>0.091 (0.000)</td>
<td>0.091 (0.000)</td>
<td>0.091 (0.000)</td>
</tr>
<tr>
<td>Sex</td>
<td>10</td>
<td>0.21 (0.08 to 0.35)</td>
<td>5.48 (0.000)</td>
<td>0.23 (0.000)</td>
<td>0.23 (0.000)</td>
<td>0.23 (0.000)</td>
</tr>
</tbody>
</table>

*InCHIANTI was excluded owing to an extremely low CC frequency of 2%.†Raine study was not included as it was conducted in Australia.

we could not separate the effect of individual dairy products in our study to further explain the inconsistency between observational and instrumental results using the current instrumental variable. And it is difficult to find a specific instrumental variable for each dairy product.

To tackle the heterogeneity of the association between SNP rs4988235 and dairy intake across studies, we conducted stratified analysis by CC frequency and region or country. SNP rs4988235 was consistently associated with higher dairy intake across subgroups, showing the robustness of our instrumental variable. No heterogeneity was found among studies conducted in northern Europe or among studies with a CC frequency of 12% or less, perhaps because these populations consume a relatively high amount of dairy products, and SNP rs4988235 was found to be associated completely with lactase persistence in north Europeans. No associations of genetically determined dairy intake with systolic blood pressure and risk of hypertension were found in both subgroups, which were consistent with our main finding.

**Conclusion**

The weak inverse association between dairy intake and systolic blood pressure in observational studies was not supported by our comprehensive instrumental variable analysis and systematic review of existing clinical trials.

Members of the Mendelian Randomization of Dairy Consumption Working Group

Ming Ding, research fellow, Tao Huang, assistant professor, Helle K M Bergholdt, research fellow, 6,7 Alex C Frazier-Wood, assistant professor, 5,6,7 Rozenn N Lemaitre, research associate professor, 1,3,5 Ester A L de Andrade, research fellow, 5,6,7,30 Shafqat Ahmad, research fellow, 1,3,32 Frida Renström, research fellow, 1,3,37 Bruce M Piatty, professor, 1,35,37,38 David S Siscovick, professor, 1,36 Barroso, senior group leader, 37,38,39 Inggeord Johansson, professor, 1,36,39 Dena Hernandez, biologist, 1,36 Luigi Ferrucci, scientific director, 2,6,39 Stefania Bandinelli, geneticist, 4,39 Allan Linneberg, professor, 1,39 Camilla Helene Sandholt, research fellow, 27 Oluf Pedersen, professor, 27,46 Torben Hansen, professor, 27,47 Christine-Alexandra Schulz, doctoral student, 46 Emily Stonedest, associate professor, 27 Marju Orho-Melander, professor, 27,48 Tzu-A Chen, senior statistician, 4,39 Jerome I Rotter, professor, 39,48 Mathew A Allison, professor, 39,48 Stephen S Rich, professor, 39,48 Jose V Sorlí, professor, 39 Oscar Collen, professor, 27,46 Craig E Pennell, professor, 39 Peter R Eastwood, professor, 4,24 Albert Hofman, professor, 27,4 Andre G Uitterlinden, professor, 1,36 M Carola Zillikens, associate professor, 27,4 Frank J A van Rooij, research associate, 27,4 Audrey Y Chu, research fellow, 27 Lyndia M Rose, associate professor, 27 Paul M Ridker, professor, 27,45,46 Olga Raitakari, project coordinator professor, 27,46 Linnea Skiöld, associate professor, 27,46 Xiaolin Yang, associate professor, 27,46 Walter C Willett, professor, 27,46 Yue Li, associate professor, 27,46 Katherine L Tucker, professor, 27 Jose M Ordovas, senior scientist, 27,4,46 Tuomas O Kieliainen, associate professor, 27,46 Michael A Province, professor, 27,46 Paul W Franks, professor, 27,46 Donna K Arnett, professor, 27,46 Toshiko Tanaka, staff scientist, 27,46 Ulla Toft, associate professor, 27,46 Ulrika Ericson, associate researcher, 27,46 Oscar H Franco, professor, 27,46 CHARGe consortium, Danush Mozafariann, professor, 27 Frank B Hu, professor, 27,46 Daniel I. Chasman, associate professor, 27,46 Barge G Nordestgaard, professor, 27,46 Christin A. Eggert, associate professor, 27,46,47 Lu Qi, professor, 1,36
The authors of the GLACIER Study acknowledge the funding agencies supporting the Northern Sweden Diet Database and the Vasterbotten Intervention Project, including the Swedish Research Council. The authors of the YFS gracefully acknowledge the expert technical assistance in the statistical analyses by Ville Aalto, Inna Lisinen, and Mika Helminen. The authors of the Raine study are grateful to the Raine participants and their families, and to the Raine research staff for cohort coordination and data collection. This work was supported by resources provided by the Pawsley Supercomputing Centre with funding from the Australian Government and the Government of Western Australia. The authors also gratefully acknowledge the NH&MRC for their long term funding to the study over the past 25 years and also the following institutes for providing funding for core management of the Raine study: University of Western Australia (UWA); Curtin University, Raine Medical Research Foundation, UWA Faculty of Medicine, Dentistry, and Health Sciences; Tel Aviv University, Institute, Women’s and Infant’s Foundation Research Fund (King Edward Memorial Hospital); and Edith Cowan University. We acknowledge the assistance of the Western Australian DNA Bank (National Health and Medical Research Council of Australia National Enabling Facility), the Raine study participants for their ongoing participation, the Raine study team for study coordination and data collection; the UWA Centre for Science for use of its facility; and the Sleep Study Technicians. The authors of the ARC study thank the staff and participants for their important contributions. Dr Dolores Corella acknowledges the collaboration of the Real Colegio Complutense at Harvard University, Cambridge, MA, USA.

Contributors: LQ obtained funding from the National Institutes of Health, MD, TH, HKB, CE, and LQ designed the study. MD and TH collected the data. MD, TH, and HKB provided statistical expertise. MD and CE analyzed the data and wrote the first draft of the manuscript. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. MD, TH, HKB, BGN, CE, and LQ are the guarantors of this investigation and contribute equally to this work.

Funding: Funding: LQ is recipient of the National Heart, Lung, and Blood Institute (HL117193, HL123495), National Institute of Diabetes and Digestive and Kidney Diseases (DK091718, DK100338, DK078616), the Boston Obesity Nutrition Research Center (DK46200), the United States—Israel Binational Science Foundation grant (2011036), and the American Heart Association Scientist Development Award (17SDG33900486). The GLACIER study was supported in part by theRegion Zealand Foundation, Naestved Hospital Foundation. Edith and Henrik Henriksens Memorial Scholarship, Johan and Lise Boserup Foundation, TrygFonden, Johannes Fog’s Foundation, Region Zealand, Naestved Hospital, National Board of Health, and Local Government Denmark Foundation. The WGIS is supported by HL043581 and HL080407 from the National Heart, Lung, and Blood Institute and CA047988 from the National Cancer Institute, the Donald W Reynolds Foundation and the Foundation Leducq, with collaborative scientific support and funding for genotyping provided by Amgen. This work was supported by grants UM1 CA186107, P01 CA87969, R01 CA49449, R01 HL034594, R01 HL088521, UM1 CA16752, R01 HL35644, HL07168, CA87969, CA44949, CA055075, HL34594, HL088521, UH1G004399, DK080140, P30, U01CA107088, USA5156626, DK58845, DK098311, U01HG004724, EY015473, CA134958, DK52870, USA5156626, and HK090 from the National Institutes of Health, with additional support for genotyping provided by Merck Research Laboratories, North Wales, PA. LRP is supported by the Arthur Ashley Williams Foundation and a Harvard ophthalmology scholar award (Harvard Medical School) from the Harvard Graduate School of Arts and Sciences. Dr Dolores Corella is supported by the American Cancer Foundation clinical investigator. The funding sources had no role in the design or conduct of the study, collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript. ARIC is carried out as a collaborative study supported by the National Heart, Lung, and Blood Institute, in collaboration with the National Institute on Aging (HHSN26820110005C, HHSN26820110006C, HHSN26820110007C, HHSN26820110008C, HHSN26820110009C, HHSN26820110000C).

doi: 10.1136/bmj.j1000 | BMJ 2017;356:j1000 | the bmj
Research Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The YFS has been financially supported by the Academy of Finland: grants 286282 (TL), 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071. (Skål.) The CHARGE Study was supported by NHLBI grants U01HL080295, R01HL087649, and R01HL086694, National Human Genome Research Institute contract U01HG004462, and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by grant No UL1TR002505, a component of the National Institutes of Health and NHLBI Roadmap for medical research. The Inter99 study was funded by the Danish Research Councils, Health Foundation, Danish Centre for Evaluation and Health Technology Assessment, Copenhagen County, Danish Heart Foundation, Ministry of Health and Prevention, Association of Danish Pharmacies, Augustinus Foundation, Novo Nordisk, Velux Foundation, Becket Foundation, and b Henriksens Foundation. The D.E.S.I.R. study has been supported by INSERM contracts with CNAMTS, Lilly, Novartis Pharma, and Sanofi-Aventis; by INSERM (Réseaux en Sante Publique, Intérêts entre les déterminants de la santé, Cohortes Santé TIGR 2008), the Association Diabète Risque Vasculaire, the Fédération Française de Cardiologie, La fondation de France, ALFEDIAN, CNIÉL, ONIVINS, Société Francophone du Diabète, Ardis Medical, Bayer Diagnostics, Becton Dickinson, Cardionics, Merck Santé, Novo Nordisk, Pierre Fabre, Roche, Topcon. The funding sources had no role in the design or conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript. The D.E.S.I.R. Study Group: INSERM CESP U1018: B Balkau, P Dumitrece, E Eschwege; INSERM U367: F Alidency-Gelas, CHU d’Angers: A Girault, Bichat Hospital: F Fumeron, M Marre, R Roussel, CHU de Rennes: F Bonnet, CNRS UMR9089, Lille: S Cauchi P Freguglione, Centers d’Examens de Santé: Alencon, Angers, Blois, Caen, Chartres, Chateauroux, Cholet, Le Mans, Orléans, Tours, Institute de Recherche Médicale Générale: J Congue; General practitioners of the region; Institute inter-Regional pour la santé: C Born, E Caces, N Copin, JG Moreau, O Lanterni, F Rale-Arnold, J Tichet, S Vol. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists. The generation and management of GWAS genotype data for the Rotterdam Study is supported by the Netherlands Organisation of Scientific Research (NWO) Investments (No 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (O14-93-015; RIDE), the Netherlands Genomics Initiative (NGI)/ Netherlands Organisation for Scientific Research (NWO) project No 050-060-810. The MDCS was initiated and planned in collaboration with the International Agency for Research on Cancer, the Swedish Cancer Society, and Swedish Medical Research Council and the Faculty of Medicine Lund University, Sweden. The study is also funded by Region Skåne, City of Malmö, Påhlsson Foundation and the Swedish Heart and Lung Foundation. The GLACIER Study was funded by project grants from the Swedish Heart-Lung Foundation, the Swedish Diabetes Association, the Påhlsson’s Foundation, Region Skåne, the Swedish Research Council, the Umeå Medical Research Foundation, Novo Nordisk, The Heart Foundation of Northern Sweden (all to PWF), and Wellcome Trust WT099051. The MESA study was supported by contracts HSNS268201000031, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 from the National Heart, Lung, and Blood Institute, and by grants UL1TR00040 and UL1-TR-001079 from NCOR. The FamHS was supported by grants DK089256 and HL170708 from the National Institutes of Health. Infrastructure for the CHARGE Consortium is supported in part by the National Heart, Lung, and Blood Institute grant R01HL105756. This CHS research was supported by NHLBI contracts HSNS268201200036C, HSNS26820000007C, N01HC55222, N01HC55079, N01HC55080, N01HC58501, N01HC58502, N01HC58503, N01HC58506, and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, and R01HL120939 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG036529 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, (grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology


**Supplementary appendix: additional information**