Mendelian randomization studies of biomarkers and type 2 diabetes

Ali Abbasi [1,2,3]

1 MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Addenbrooke's Hospital, P.O. Box 285, Cambridge CB2 0QQ, UK

2 Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

3 Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

Short title: Biomarkers and type 2 diabetes

Number of Tables: 2

Number of Figures: 1

Correspondence: Dr. Ali Abbasi, MD, PhD, MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Addenbrooke's Hospital, P.O. Box 285, CB2 0QQ, Cambridge, the United Kingdom

Tel: +44 1223 769212; Fax: +44 1223 330316; e-mail: ali.abbasi@mrc-epid.cam.ac.uk

Abstract

CONTEXT: Many biomarkers are associated with type 2 diabetes (T2D) risk in epidemiological observations. The aim of this study was to identify and summarize current evidence for causal effects of biomarkers on T2D.

DESIGN AND METHODS: A systematic literature search in PubMed and EMBASE (until April 2015) was done to identify Mendelian randomization studies that examined potential causal effects of biomarkers on T2D. To replicate the findings of identified studies, data from two large-scale genome-wide association studies (GWAS) were used: 1) DIAbetes Genetics Replication And Meta-analysis (DIAGRAMv3) for T2D, and 2) the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) for glycaemic traits. GWAS summary statistics were extracted for the same genetic variants (or proxy of variants) which were used in the original Mendelian randomization studies.

RESULTS: Ten out of 21 biomarkers (from 28 studies) have been reported to be causally associated with T2D in Mendelian randomization. Most biomarkers were investigated in a single cohort study or population. Of the 10 biomarkers that were identified, nominally significant associations with T2D or glycaemic traits were reached for those genetic variants related to bilirubin, pro-B-type natriuretic peptide, Delta-6 desaturase and dimethylglycine based on the summary data from DIAGRAMv3 or MAGIC.

CONCLUSIONS: Several Mendelian randomization studies investigated the nature of associations of biomarkers with T2D. However, there were only few biomarkers that may have causal effects on T2D. Further research is needed to broadly evaluate the causal effects of multiple biomarkers on T2D and glycaemic traits using data from large-scale cohorts or GWAS including many different genetic variants.

Keywords: Biomarkers, Epidemiology, Mendelian randomization, Genome-wide association study, Type 2 diabetes

Abbreviations: BNP= B-type natriuretic peptide; D6D= Delta-6-desaturase; DIAGRAM= DIAbetes Genetics Replication And Meta-analysis; GWAS=Genome-wide association studies; MAGIC= Meta-Analyses of Glucose and Insulin-related traits Consortium; MIF= macrophage migration inhibitory factor; SHBG= sex hormone binding protein

Introduction

Over the past decade, much interest in studying biological markers (biomarkers) for type 2 diabetes (T2D) has been attracted. This happened because multiple pathobiological processes may contribute to the disease progression, which provides an opportunity to introduce preventive and therapeutic interventions for T2D [1]. In clinical practice, such biomarkers (e.g., glucose and glycated haemoglobin tests) are widely used for diagnosis of diabetes or for monitoring of therapeutic intervention [2, 3].Targeted intervention at biomarker level would be useful where there is evidence for a causal relationship between an exposure (like a biomarker) and T2D [4].

Traditional epidemiological studies lack sufficient information to fill the evidence gap due to unmeasured confounding or reverse causality[4-7]. It has been successfully shown that a complementary analysis of genetic data, termed "Mendelian randomization", has additive value to infer a causal association[4-8]. The main assumption for Mendelian randomization is that the genetic variants do not change over time and are inherited randomly (based on Mendel's laws). In other words, the genetic variants as proxy measures for exposures (e.g., biomarkers) are essentially considered free from confounding and reverse causation. Therefore, the analysis of integrated observational-genetic data is considered similar to that of the randomized trials[9, 10]. In Mendelian randomization, if there is a causal association between a biomarker and T2D, the genetic variant(s) influencing biomarker and the outcome of interest should be associated [5, 6, 8]. In the current study, evidence for causal associations between biomarkers and the risk of T2D was updated via a systematic literature search to identify Mendelian randomization studies. Next, summary data from two largest genome-wide association studies (GWAS) for T2D or glycaemic traits[10, 11] were used to examine the effect estimates for each genetic variants compared with that of the identified studies.

Methods

Search strategy for candidate biomarkers

PubMed and EMBASE were searched to identify Mendelian randomization studies examining the associations between biomarkers and T2D until April 2015. The overview of this systematic literature search was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines, when applicable[12]. A manual search was also done for the references of included articles to identify other relevant studies. Because a review of published original studies was performed, the Declaration of Helsinki items related to "approval of medical ethics committee" and "permission acquisition" are not applicable to the current study.

Selection criteria

Studies were included if: 1) they formally quantified a causal association between one or more biomarkers (as main exposures) and T2D (as the main outcome); 2) used data on biomarker-associated genetic variants: 3) and classified/defined the exposure as a biomarker that has been objectively measured in serum, plasma or urine. The following MeSH key words or related terms were used: "Diabetes mellitus, Type 2 [Mesh]", Type 2 Diabetes[tiab], Diabetes[ti]; and "Mendelian randomization", "Mendelian randomisation", "instrumental variable", "genetic risk score" or "causal association".

Data extraction and quality assessment

A primary plan was made to extract necessary data from the full text of the original studies or to contact the corresponding author(s) when appropriate. Figure 1depicts the work-flow of the literature search. T2D was determined as the main outcome if one or more of the following conditions were fulfilled: 1) a physician diagnosed T2D as indicated by self-report

104 or in primary-care database; 2) fasting plasma glucose ≥7.0 mmol/l, a random sample plasma
105 glucose ≥11.1 mmol/l; or 3) initiation of glucose-lowering medication as retrieved from a
106 pharmacy registry or hospital records[11].
107
108 *Statistical analysis*
109 All genetic variants that affect identified biomarkers were obtained from the original
110 Mendelian randomization studies. To replicate the nature of relationship between each
111 biomarker and the outcome (i.e., T2D or glycaemic traits), a genetic approach using outcome-
112 association data for the biomarker-related genetic variants was applied [5, 6, 8]. In brief, to
113 determine whether the same genetic variants (or a suitable proxy variant) were associated with
114 T2D (or glycaemic traits), the corresponding summary association statistics from GWAS for
115 T2D (DIAbetes Genetics Replication And Meta-analysis [DIAGRAMv3])[13] and glycaemic
116 traits (Meta-Analyses of Glucose and Insulin-related traits Consortium [MAGIC]) [14]were
117 extracted. DIAGRAMv3 is a meta-analysis of multiple GWAS with a total number of 12,171
118 diabetes cases and 56,862 controls of European descent[13]. Previously, details regarding the
119 use of GWAS data for Mendelian randomization were described[5, 6, 10, 11]. These selected
120 single-test association analyses in the GWAS data are equivalent to that of individual-level
121 data analysis [5, 6, 11].
122 Extracted data were tabulated on Excel spreadsheets. All statistical analyses were
123 conducted using Excel or Stata/SE version 13.1 for Windows (http://cran.r-project.org/). A
124 two-sided *P* value<0.05 was considered nominally significant.
125

Results

Literature search and study characteristics

After scanning 812 titles and selected abstracts, 33 articles were selected for full text review [11, 15-46]. The literature search process is shown in Figure 1. Five studies were excluded as they did not measure a specific biomarker (n=1) or investigated a measure of insulin resistance as the main exposure (n=4)[15, 38-40, 43]. The characteristics of the 28 studies are summarized in Table 1.The studies were performed in different populations; most were cohort studies included middle-aged adults in the US or Europe and were published between 2008-2015. Four studies were conducted only in Asian populations including Chinese or Taiwanese. In 12 studies, data from multiple GWAS were combined to examine the associations between genotypes and T2D. Six of these studies used data from DIAGRAMv2 (n=3)[47] or DIAGRAMv3 (n=3)[13], and the other six used at least two cohorts with genome-wide genotyping. DIAGRAMv2 was a meta-analysis of eight GWAS comprising 8,130 individuals with T2D and 38,987 controls of European descent[47]. All Mendelian randomization studies investigated only one biomarker as an exposure in relation to T2D. Some studies also examined the causal associations of a single biomarker with several other outcomes such as cardiovascular disease[18, 24], rheumatoid arthritis[18] or osteoporosis[24]. In the final sample, studies included up to 28144/76344 T2D cases/controls or total participants.

Biomarkers and Mendelian randomization

From 28 studies, data were retrieved on causal associations of biomarkers with T2D. In these studies, 21 unique biomarkers that were investigated at least once (16 biomarkers), twice (three biomarkers) and three times (two biomarkers) were identified (Table 1). Nineteen biomarkers were completely investigated in independent studies. However, the two biomarkers with three Mendelian randomization studies were investigated in combined

studies where the same sample , e.g. DIAGRAMv2 for adiponectin[19, 43], or a part of whole cohort, e.g. EPIC-Potsdam for vitamin D[17, 45], were used to make total cases/controls. Eleven studies used one genetic variant as a single instrumental variable in Mendelian randomization. Eight studies used at least two independent genetic variants in the same locus as instrumental variables. The rest of nine studies used multiple genetic variants in different loci or created a multi-locus genetic risk score for each biomarker.

In main or sensitivity analyses, four studies [11, 26, 44, 45] examined causal associations of bilirubin, Lipoprotein(a), vitamin D, interleukin 1 receptor antagonist (with corresponding biomarker-associated genotypes) with type 2 diabetes using the DIAGRAMv3 summary data. In Table 1, the value for each causal estimate can be interpreted as odds ratio (OR), hazard ratio (HR) or log OR (β coefficient) for diabetes per one unit change in genetically determined biomarkers or biomarker-associated genotypes. Evidence of causal association was reported for adiponectin, bilirubin, N-terminal pro B-type natriuretic peptide (NT proBNP), Delta-6 desaturase (D6D), dimethylglycine, ferritin (Transmembrane protease serine 6), homocysteine, macrophage migration inhibitory factor (MIF), sex hormone binding protein (SHBG) and resistin (Table 1). For adiponectin, causal estimates were calculated as an OR of 0.86 per allele[35] or a β coefficient of 0.3 for adiponectin multi-locus genotypic risk score [19]. Causal estimate for bilirubin was reported as an OR of 0.58 per 1-SD genetically increased log-transformed bilirubin[11]. For NT proBNP[37], D6D[30], dimethylglycine[32], ferritin[22], MIF[23] and resistin[18], the expected ORs for T2D were 0.96, 0.64, 1.1, 0.79, 1.74 and 1.38 per each copy of risk alleles or genotypes, respectively. For homocysteine, causal estimates were reported as an OR of 1.29 per 5μmol/L genetically increased homocysteine[24], or ORs of 1.93 and 1.52 for CC and CC/TT genotypes[25], respectively. For SHBG, the expected ORs for T2D were 0.92 per each copy of the SHBG lowering allele[34] or 0.3 per 1-SD genetically increased natural log-transformed in SHBG[21].

176 Using the GWAS data from DIAGRAMv3 or MAGIC [13, 14], associations of the genetic
177 variants influencing these biomarkers with T2D and glycaemic traits were tested. A nominally
178 significance (P <0.05) was reached for the genetic variants that affect bilirubin (P=0.03 for
179 glucose and HOMA-IR), NT proBNP (P=0.03 for T2D), D6D activity (P=0.003 for T2D;
180 $P=2.7\times10^{-8}$ for glucose) and dimethylglycine (P=0.004 for glucose) in relation to T2D or
181 glycaemic traits (Table 2). For adiponectin, a nominally significant association with T2D or
182 glycaemic traits was observed for seven out of 19 genetic variants. All these seven variants,
183 except rs12637534, were non-*ADIPOQ* adiponectin genetic variants . In line with previous
184 Mendelian randomization studies, the overall effect of non-*ADIPOQ* variants on T2D or
185 glycaemic traits together with null association of *ADIPOQ* variants is compatible with
186 pleiotropic effects of adiponectin on T2D[42].
187

Discussion

This literature search of Mendelian randomization studies shows here that 10 out of 21 identified biomarkers were reported to be causally associated with T2D. In particular, the presence of potential causal associations (defined as nominally significant) between the biomarker-related variants and T2D and/or glycaemic traits can be confirmed for four biomarkers using the publically-available GWAS data. The inconsistency between the identified studies and the summary data from DIAGRAMv3 or MAGIC can be to some extent explained by different design and populations applying across studies, the possibility of false positive associations, the heterogeneity in T2D, the use of varied sources to ascertain T2D cases, and differences in data quality control and pre-analysis preparations [6, 11].

Taken together, these findings support that the oxidative stress system (bilirubin or metabolites of bilirubin), the BNP hormone system, D6D activity, and dimethylglycine may contribute to the development of diabetes or insulin resistance through secondary effects (i.e., pleiotropy) or direct mechanisms[11, 37, 42]. Bilirubin that is the major end-product of heme catabolism has antioxidant properties and may compensate the oxidative stress[11, 48]. Oxidative stress has been shown as an important factor in the pathophysiology of diabetes[11]. At the cellular level, bilirubin can be oxidized to its precursor, biliverdin, to detoxify excess of oxidants. Biliverdin is rapidly recycled to bilirubin via the action of biliverdin reductase, generating a physiologic cytoprotective cycle in several tissues[49]. The underlying mechanism of a protective role of BNP in the aetiology of T2D is unknown in humans[37]. In mice, over-expressed BNP signalling cascade can protect against diet-induced insulin resistance and obesity through promoting muscle mitochondrial biogenesis and fat oxidation[37, 50]. The biological mechanisms of the relationship between D6D activity T2D are not well understood[30]. Although data of human experimental studies are scarce, observational studies have shown that D6D activity or lifestyle-induced changes in D6D activity was associated

213 with insulin resistance[30, 51, 52]. Since D6D catalyses the synthesis of fatty acids, one can
214 speculate that the link between D6D activity and T2D is likely to be mediated by changes in
215 fatty acid composition, which in turn may affect insulin signalling and receptor binding
216 affinities[30]. Dimethylglycine is metabolized to glycine by dimethylglycine dehydrogenase
217 (DMGDH) in mammals[32]. Accordingly, a recent GWAS identified that the *DMGDH* genetic
218 variants were strongly associated with blood-based dimethylglycine[53]. Epidemiological
219 studies have observed an inverse association between the precursor of dimethylglycine –
220 betaine – and metabolic risk factors[54], but a positive association of elevated glycine with
221 increased insulin sensitivity[32]. In humans, cardiometabolic effects of inhibition of DMHDH or
222 supplementation of dimethylglycine have not been investigated[32]. However, experimental
223 animal studies have suggested a protective role of dimethylglycine in glucose metabolism
224 through reduction in DMGDH function[32, 55, 56].

225 In the post-omics era, epidemiological studies basically suggest that the levels of a
226 given biomarker differ between patients with T2D (or the individuals at high risk for
227 developing diabetes) and individuals without diabetes [2, 57]. If the biomarker is not causally
228 related to the disease outcome, the process of developing diabetes may cause the increase or
229 decrease in the levels of biomarker, as one of the T2D consequences, called reverse causality[4,
230 26, 37]. Unmeasured confounding or measured confounding factors with errors (for example,
231 physical activity by self-report) is another explanation for the observed associations between
232 most biomarkers and T2D. In this context, the use of genetic data (like in Mendelian
233 randomization) can enhance the likelihood that association between a biomarker and T2D is
234 causal or not, where biomarkers are subject to the evaluation of causal inference [4, 36, 37]. It
235 remains to be further confirmed for the potential role of biomarkers in the development of
236 T2D and the trajectories of glycaemic traits using longitudinal analysis [6, 11]. The latter
237 analytical approach can provide insight into potential value of biomarkers which indicate

pathobiological signals of metabolic changes in the aetiology of T2D. T2D is influenced by interactions of multiple genes or a gene may have been mapped to multiple biomarkers other than the biomarker of interest [4, 13, 14]. Thus, an extensive knowledge of gene function, biological processes and that the genome interacts with environmental factors is needed to better understand how genetic variations in the human genome contributes to lifelong risk of T2D [4, 11, 13, 14, 57].

In this review, most studies only investigated a single biomarker-diabetes association and statistically significant associations are reported more often. Here publication bias should be considered. Other limitations include lack of complementary analyses in the genetic associations for glycaemic traits, and that set of genetic variants or large-scale GWAS for biomarkers were scare. Similarly, the Mendelian randomization approach using GWAS summary statistics can be extended as a secondary analysis of several datasets that have data on the biomarker-associated variants (or their proxies) for several diseases or traits. This multi-disciplinary research requires that a large group of consortia are contacted to obtain summary association statistics from GWAS consortia for outcomes of interest. Moreover, for the biomarkers linked to T2D, underlying biological mechanisms remain unknown. To uncover the underlying mechanisms, one needs to perform a complementary strand of experimental research. Before that, an *in-silico* functional gene network (pathway) analysis can be used to speculate on the possible biological mechanisms of biomarker-disease associations[58, 59]. Finally, Mendelian randomization cannot completely control for the possibility of developmental compensation, called canalization, confounding and pleiotropic effects [5, 6, 8, 11, 37].

In conclusion, this is the first study that updates evidence for causal associations between biomarkers and T2D to date. Several Mendelian randomization studies investigated the nature of associations of biomarkers with T2D. Most biomarkers were investigated in a single cohort

study or population. However, there are only few biomarkers that may have causal effects on T2D. Further research is warranted to broadly evaluate the causal effects of multiple biomarkers on T2D and glycaemic traits using data from large-scale cohorts or combined GWAS including many different genetic variants. This genetic approach may advance our understanding of the causes of T2D, and potentially enable us to explore novel targets for the prevention and treatment of diabetes.

Research funding: This work was supported by the Netherlands Organization for Scientific Research project (NWO), and the Medical Research Council UK (grant no. MC_UU_12015/1). AA is supported by a Rubicon grant from the NWO (Project no. 825.13.004).

Author' conflict of interest disclosure: The author has nothing to disclose. None of the study sponsors had a role in the study design, data collection, analysis and interpretation, report writing, or the decision to submit the report for publication. The author declares no conflict of interest, no financial relationships with any organizations that might have an interest in the submitted work, no other relationships or activities that could appear to have influenced the submitted work.

References:

1. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM & Diabetes Prevention Program Research G. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002 **346** 393-403.
2. Abbasi A, Stolk RP & Bakker SJ. Identification of relevant biomarkers for type 2 diabetes. *Lancet Diabetes Endocrinol* 2014 **2** 106-107.
3. Lyons TJ & Basu A. Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers. *Transl Res* 2012 **159** 303-312.
4. Sandhu MS, Debenham SL, Barroso I & Loos RJ. Mendelian randomisation studies of type 2 diabetes: future prospects. *Diabetologia* 2008 **51** 211-213.
5. Nelson CP, Hamby SE, Saleheen D, Hopewell JC, Zeng L, Assimes TL, Kanoni S, Willenborg C, Burgess S, Amouyel P, Anand S, Blankenberg S, Boehm BO, Clarke RJ, Collins R, Dedoussis G, Farrall M, Franks PW, Groop L, Hall AS, Hamsten A, Hengstenberg C, Hovingh GK, Ingelsson E, Kathiresan S, Kee F, Konig IR, Kooner J, Lehtimaki T, Marz W, McPherson R, Metspalu A, Nieminen MS, O'Donnell CJ, Palmer CN, Peters A, Perola M, Reilly MP, Ripatti S, Roberts R, Salomaa V, Shah SH, Schreiber S, Siegbahn A, Thorsteinsdottir U, Veronesi G, Wareham N, Willer CJ, Zalloua PA, Erdmann J, Deloukas P, Watkins H, Schunkert H, Danesh J, Thompson JR, Samani NJ & Consortium CACD. Genetically Determined Height and Coronary Artery Disease. *N Engl J Med* 2015.
6. VanderWeele TJ, Tchetgen Tchetgen EJ, Cornelis M & Kraft P. Methodological challenges in mendelian randomization. *Epidemiology* 2014 **25** 427-435.
7. Nikolopoulos GK, Bagos PG, Tsangaris I, Tsiara CG, Kopterides P, Vaiopoulos A, Kapsimali V, Bonovas S & Tsantes AE. The association between plasminogen activator inhibitor type 1 (PAI-1) levels, PAI-1 4G/5G polymorphism, and myocardial infarction: a Mendelian randomization meta-analysis. *Clin Chem Lab Med* 2014 **52** 937-950.
8. Haase CL, Tybjaerg-Hansen A, Qayyum AA, Schou J, Nordestgaard BG & Frikke-Schmidt R. LCAT, HDL cholesterol and ischemic cardiovascular disease: a Mendelian randomization study of HDL cholesterol in 54,500 individuals. *J Clin Endocrinol Metab* 2012 **97** E248-256.
9. Smith GD & Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol* 2004 **33** 30-42.
10. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG & Consortium E-I. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol* 2015.
11. Abbasi A, Deetman PE, Corpeleijn E, Gansevoort RT, Gans RO, Hillege HL, van der Harst P, Stolk RP, Navis G, Alizadeh BZ & Bakker SJ. Bilirubin as a potential causal factor in type 2 diabetes risk: a mendelian randomization study. *Diabetes* 2015 **64** 1459-1469.
12. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J & Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009 **339** b2700.
13. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, Prokopenko I, Kang HM, Dina C, Esko T, Fraser RM, Kanoni S, Kumar A, Lagou V, Langenberg C, Luan J, Lindgren CM, Muller-Nurasyid M, Pechlivanis S, Rayner NW, Scott LJ, Wiltshire S, Yengo L, Kinnunen L, Rossin EJ, Raychaudhuri S, Johnson AD, Dimas AS, Loos RJ, Vedantam S, Chen H, Florez JC, Fox C, Liu CT, Rybin D, Couper DJ, Kao WH, Li M, Cornelis MC, Kraft P, Sun Q, van Dam RM, Stringham HM, Chines PS, Fischer K, Fontanillas P, Holmen OL, Hunt SE, Jackson AU, Kong A, Lawrence R, Meyer J, Perry JR, Platou CG, Potter S, Rehnberg E, Robertson N, Sivapalaratnam S, Stancakova A, Stirrups K, Thorleifsson G, Tikkanen E, Wood AR, Almgren P, Atalay M, Benediktsson R, Bonnycastle LL, Burtt N, Carey J, Charpentier G, Crenshaw AT, Doney AS, Dorkhan M, Edkins S, Emilsson V, Eury E, Forsen T, Gertow K, Gigante B, Grant GB, Groves CJ, Guiducci C, Herder C, Hreidarsson AB, Hui J, James A, Jonsson A, Rathmann W, Klopp N, Kravic J, Krjutskov K, Langford C, Leander K, Lindholm E, Lobbens S, Mannisto S, Mirza G, Muhleisen TW, Musk B, Parkin M, Rallidis L, Saramies J, Sennblad B, Shah S, Sigurethsson G, Silveira A, Steinbach G, Thorand B, Trakalo J, Veglia F, Wennauer R, Winckler W, Zabaneh D, Campbell H, van Duijn C, Uitterlinden AG, Hofman A, Sijbrands E, Abecasis GR, Owen KR, Zeggini E, Trip MD, Forouhi NG, Syvanen AC, Eriksson JG, Peltonen L, Nothen MM, Balkau B, Palmer CN, Lyssenko V, Tuomi T, Isomaa B, Hunter DJ, Qi L, Wellcome Trust

Case Control C, Meta-Analyses of G, Insulin-related traits Consortium I, Genetic Investigation of ATC, Asian Genetic Epidemiology Network-Type 2 Diabetes C, South Asian Type 2 Diabetes C, Shuldiner AR, Roden M, Barroso I, Wilsgaard T, Beilby J, Hovingh K, Price JF, Wilson JF, Rauramaa R, Lakka TA, Lind L, Dedoussis G, Njolstad I, Pedersen NL, Khaw KT, Wareham NJ, Keinanen-Kiukaanniemi SM, Saaristo TE, Korpi-Hyovalti E, Saltevo J, Laakso M, Kuusisto J, Metspalu A, Collins FS, Mohlke KL, Bergman RN, Tuomilehto J, Boehm BO, Gieger C, Hveem K, Cauchi S, Froguel P, Baldassarre D, Tremoli E, Humphries SE, Saleheen D, Danesh J, Ingelsson E, Ripatti S, Salomaa V, Erbel R, Jockel KH, Moebus S, Peters A, Illig T, de Faire U, Hamsten A, Morris AD, Donnelly PJ, Frayling TM, Hattersley AT, Boerwinkle E, Melander O, Kathiresan S, Nilsson PM, Deloukas P, Thorsteinsdottir U, Groop LC, Stefansson K, Hu F, Pankow JS, Dupuis J, Meigs JB, Altshuler D, Boehnke M, McCarthy MI, Replication DIG & Meta-analysis C. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet* 2012 **44** 981-990.

14. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Magi R, Morris AP, Randall J, Johnson T, Elliott P, Rybin D, Thorleifsson G, Steinthorsdottir V, Henneman P, Grallert H, Dehghan A, Hottenga JJ, Franklin CS, Navarro P, Song K, Goel A, Perry JR, Egan JM, Lajunen T, Grarup N, Sparso T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proenca C, Kumari M, Qi L, Timpson NJ, Gieger C, Zabena C, Rocheleau G, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Payne F, Roccasecca RM, Pattou F, Sethupathy P, Ardlie K, Ariyurek Y, Balkau B, Barter P, Beilby JP, Ben-Shlomo Y, Benediktsson R, Bennett AJ, Bergmann S, Bochud M, Boerwinkle E, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Bottcher Y, Brunner E, Bumpstead SJ, Charpentier G, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Cornelis M, Crawford G, Crisponi L, Day IN, de Geus EJ, Delplanque J, Dina C, Erdos MR, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Fox CS, Frants R, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Groves CJ, Grundy S, Gwilliam R, Gyllensten U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanali N, Hayward C, Heath SC, Hercberg S, Herder C, Hicks AA, Hillman DR, Hingorani AD, Hofman A, Hui J, Hung J, Isomaa B, Johnson PR, Jorgensen T, Jula A, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimaki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoeur C, Li Y, Lyssenko V, Mahley R, Mangino M, Manning AK, Martinez-Larrad MT, McAteer JB, McCulloch LJ, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Morken MA, Mukherjee S, Naitza S, Narisu N, Neville MJ, Oostra BA, Orru M, Pakyz R, Palmer CN, Paolisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Perola M, Pfeiffer AF, Pichler I, Polasek O, Posthuma D, Potter SC, Pouta A, Province MA, Psaty BM, Rathmann W, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Roden M, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Scott LJ, Seedorf U, Sharp SJ, Shields B, Sigurethsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvanen AC, Tanaka T, Thorand B, Tichet J, Tonjes A, Tuomi T, Uitterlinden AG, van Dijk KW, van Hoek M, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Walters GB, Ward KL, Watkins H, Weedon MN, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC, Consortium D, Consortium G, Global BC, Borecki IB, Loos RJ, Meneton P, Magnusson PK, Nathan DM, Williams GH, Hattersley AT, Silander K, Salomaa V, Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Rios M, Morris AD, Lind L, Palmer LJ, Hu FB, Franks PW, Ebrahim S, Marmot M, Kao WH, Pankow JS, Sampson MJ, Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP, Wichmann HE, Illig T, Rudan I, Wright AF, Stumvoll M, Campbell H, Wilson JF, Anders Hamsten on behalf of Procardis C, investigators M, Bergman RN, Buchanan TA, Collins FS, Mohlke KL, Tuomilehto J, Valle TT, Altshuler D, Rotter JI, Siscovick DS, Penninx BW, Boomsma DI, Deloukas P, Spector TD, Frayling TM, Ferrucci L, Kong A, Thorsteinsdottir U, Stefansson K, van Duijn CM, Aulchenko YS, Cao A, Scuteri A, Schlessinger D, Uda M, Ruokonen A, Jarvelin MR, Waterworth DM, Vollenweider P, Peltonen L, Mooser V, Abecasis GR, Wareham NJ, Sladek R, Froguel P, Watanabe RM, Meigs JB, Groop L, Boehnke M, McCarthy MI, Florez JC & Barroso I. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 2010 **42** 105-116.

15. Besseling J, Kastelein JJ, Defesche JC, Hutten BA & Hovingh GK. Association between familial hypercholesterolemia and prevalence of type 2 diabetes mellitus. *JAMA* 2015 **313** 1029-1036.

16. Brunner EJ, Kivimaki M, Witte DR, Lawlor DA, Davey Smith G, Cooper JA, Miller M, Lowe GD, Rumley A, Casas JP, Shah T, Humphries SE, Hingorani AD, Marmot MG, Timpson NJ & Kumari M. Inflammation, insulin resistance, and diabetes--Mendelian randomization using CRP haplotypes points upstream. *PLoS Med* 2008 **5** e155.
17. Buijsse B, Boeing H, Hirche F, Weikert C, Schulze MB, Gottschald M, Kuhn T, Katzke VA, Teucher B, Dierkes J, Stangl GI & Kaaks R. Plasma 25-hydroxyvitamin D and its genetic determinants in relation to incident type 2 diabetes: a prospective case-cohort study. *Eur J Epidemiol* 2013 **28** 743-752.
18. Chung CM, Lin TH, Chen JW, Leu HB, Yin WH, Ho HY, Sheu SH, Tsai WC, Chen JH, Lin SJ & Pan WH. Common quantitative trait locus downstream of RETN gene identified by genome-wide association study is associated with risk of type 2 diabetes mellitus in Han Chinese: a Mendelian randomization effect. *Diabetes Metab Res Rev* 2014 **30** 232-240.
19. Dastani Z, Hivert MF, Timpson N, Perry JR, Yuan X, Scott RA, Henneman P, Heid IM, Kizer JR, Lyytikainen LP, Fuchsberger C, Tanaka T, Morris AP, Small K, Isaacs A, Beekman M, Coassin S, Lohman K, Qi L, Kanoni S, Pankow JS, Uh HW, Wu Y, Bidulescu A, Rasmussen-Torvik LJ, Greenwood CM, Ladouceur M, Grimsby J, Manning AK, Liu CT, Kooner J, Mooser VE, Vollenweider P, Kapur KA, Chambers J, Wareham NJ, Langenberg C, Frants R, Willems-Vandijk K, Oostra BA, Willems SM, Lamina C, Winkler TW, Psaty BM, Tracy RP, Brody J, Chen I, Viikari J, Kahonen M, Pramstaller PP, Evans DM, St Pourcain B, Sattar N, Wood AR, Bandinelli S, Carlson OD, Egan JM, Bohringer S, van Heemst D, Kedenko L, Kristiansson K, Nuotio ML, Loo BM, Harris T, Garcia M, Kanaya A, Haun M, Klopp N, Wichmann HE, Deloukas P, Katsareli E, Couper DJ, Duncan BB, Kloppenburg M, Adair LS, Borja JB, Consortium D, Consortium M, Investigators G, Mu TC, Wilson JG, Musani S, Guo X, Johnson T, Semple R, Teslovich TM, Allison MA, Redline S, Buxbaum SG, Mohlke KL, Meulenbelt I, Ballantyne CM, Dedoussis GV, Hu FB, Liu Y, Paulweber B, Spector TD, Slagboom PE, Ferrucci L, Jula A, Perola M, Raitakari O, Florez JC, Salomaa V, Eriksson JG, Frayling TM, Hicks AA, Lehtimaki T, Smith GD, Siscovick DS, Kronenberg F, van Duijn C, Loos RJ, Waterworth DM, Meigs JB, Dupuis J, Richards JB, Voight BF, Scott LJ, Steinthorsdottir V, Dina C, Welch RP, Zeggini E, Huth C, Aulchenko YS, Thorleifsson G, McCulloch LJ, Ferreira T, Grallert H, Amin N, Wu G, Willer CJ, Raychaudhuri S, McCarroll SA, Hofmann OM, Segre AV, van Hoek M, Navarro P, Ardlie K, Balkau B, Benediktsson R, Bennett AJ, Blagieva R, Boerwinkle E, Bonnycastle LL, Bostrom KB, Bravenboer B, Bumpstead S, Burtt NP, Charpentier G, Chines PS, Cornelis M, Crawford G, Doney AS, Elliott KS, Elliott AL, Erdos MR, Fox CS, Franklin CS, Ganser M, Gieger C, Grarup N, Green T, Griffin S, Groves CJ, Guiducci C, Hadjadj S, Hassanali N, Herder C, Isomaa B, Jackson AU, Johnson PR, Jorgensen T, Kao WH, Kong A, Kraft P, Kuusisto J, Lauritzen T, Li M, Lieverse A, Lindgren CM, Lyssenko V, Marre M, Meitinger T, Midthjell K, Morken MA, Narisu N, Nilsson P, Owen KR, Payne F, Petersen AK, Platou C, Proenca C, Prokopenko I, Rathmann W, Rayner NW, Robertson NR, Rocheleau G, Roden M, Sampson MJ, Saxena R, Shields BM, Shrader P, Sigurdsson G, Sparso T, Strassburger K, Stringham HM, Sun Q, Swift AJ, Thorand B, Tichet J, Tuomi T, van Dam RM, van Haeften TW, van Herpt T, van Vliet-Ostaptchouk JV, Walters GB, Weedon MN, Wijmenga C, Witteman J, Bergman RN, Cauchi S, Collins FS, Gloyn AL, Gyllensten U, Hansen T, Hide WA, Hitman GA, Hofman A, Hunter DJ, Hveem K, Laakso M, Morris AD, Palmer CN, Rudan I, Sijbrands E, Stein LD, Tuomilehto J, Uitterlinden A, Walker M, Watanabe RM, Abecasis GR, Boehm BO, Campbell H, Daly MJ, Hattersley AT, Pedersen O, Barroso I, Groop L, Sladek R, Thorsteinsdottir U, Wilson JF, Illig T, Froguel P, van Duijn CM, Stefansson K, Altshuler D, Boehnke M, McCarthy MI, Soranzo N, Wheeler E, Glazer NL, Bouatia-Naji N, Magi R, Randall J, Elliott P, Rybin D, Dehghan A, Hottenga JJ, Song K, Goel A, Lajunen T, Doney A, Cavalcanti-Proenca C, Kumari M, Timpson NJ, Zabena C, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Roccasecca RM, Pattou F, Sethupathy P, Ariyurek Y, Barter P, Beilby JP, Ben-Shlomo Y, Bergmann S, Bochud M, Bonnefond A, Borch-Johnsen K, Bottcher Y, Brunner E, Bumpstead SJ, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Crisponi L, Day IN, de Geus EJ, Delplanque J, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Grundy S, Gwilliam R, Hallmans G, Hammond N, Han X, Hartikainen AL, Hayward C, Heath SC, Hercberg S, Hillman DR, Hingorani AD, Hui J, Hung J, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimaki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoeur C, Li Y, Mahley R, Mangino M, Martinez-Larrad MT, McAteer JB, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Mukherjee S, Naitza S, Neville MJ, Orru M, Pakyz R, Paolisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Pfeiffer AF, Pichler I, Polasek O, Posthuma D,

Potter SC, Pouta A, Province MA, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Seedorf U, Sharp SJ, Shields B, Sigurethsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvanen AC, Tonjes A, Uitterlinden AG, van Dijk KW, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Ward KL, Watkins H, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC, Consortium D, Consortium G, Global BPC, Borecki IB, Meneton P, Magnusson PK, Nathan DM, Williams GH, Silander K, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Serrano-Rios M, Lind L, Palmer LJ, Hu FBs, Franks PW, Ebrahim S, Marmot M, Kao WH, Pramstaller PP, Wright AF, Stumvoll M, Hamsten A, Procardis C, Buchanan TA, Valle TT, Rotter JI, Penninx BW, Boomsma DI, Cao A, Scuteri A, Schlessinger D, Uda M, Ruokonen A, Jarvelin MR, Peltonen L, Mooser V, Sladek R, investigators M, Consortium G, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Chasman DI, Johansen CT, Fouchier SW, Peloso GM, Barbalic M, Ricketts SL, Bis JC, Feitosa MF, Orho-Melander M, Melander O, Li X, Li M, Cho YS, Go MJ, Kim YJ, Lee JY, Park T, Kim K, Sim X, Ong RT, Croteau-Chonka DC, Lange LA, Smith JD, Ziegler A, Zhang W, Zee RY, Whitfield JB, Thompson JR, Surakka I, Spector TD, Smit JH, Sinisalo J, Scott J, Saharinen J, Sabatti C, Rose LM, Roberts R, Rieder M, Parker AN, Pare G, O'Donnell CJ, Nieminen MS, Nickerson DA, Montgomery GW, McArdle W, Masson D, Martin NG, Marroni F, Lucas G, Luben R, Lokki ML, Lettre G, Launer LJ, Lakatta EG, Laaksonen R, Kyvik KO, Konig IR, Khaw KT, Kaplan LM, Johansson A, Janssens AC, Igl W, Hovingh GK, Hengstenberg C, Havulinna AS, Hastie ND, Harris TB, Haritunians T, Hall AS, Groop LC, Gonzalez E, Freimer NB, Erdmann J, Ejebe KG, Doring A, Dominiczak AF, Demissie S, Deloukas P, de Faire U, Crawford G, Chen YD, Caulfield MJ, Boekholdt SM, Assimes TL, Quertermous T, Seielstad M, Wong TY, Tai ES, Feranil AB, Kuzawa CW, Taylor HA, Jr., Gabriel SB, Holm H, Gudnason V, Krauss RM, Ordovas JM, Munroe PB, Kooner JS, Tall AR, Hegele RA, Kastelein JJ, Schadt EE, Strachan DP, Reilly MP, Samani NJ, Schunkert H, Cupples LA, Sandhu MS, Ridker PM, Rader DJ & Kathiresan S. Novel loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. *PLoS Genet* 2012 **8** e1002607.

20. De Silva NM, Freathy RM, Palmer TM, Donnelly LA, Luan J, Gaunt T, Langenberg C, Weedon MN, Shields B, Knight BA, Ward KJ, Sandhu MS, Harbord RM, McCarthy MI, Smith GD, Ebrahim S, Hattersley AT, Wareham N, Lawlor DA, Morris AD, Palmer CN & Frayling TM. Mendelian randomization studies do not support a role for raised circulating triglyceride levels influencing type 2 diabetes, glucose levels, or insulin resistance. *Diabetes* 2011 **60** 1008-1018.

21. Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM & Liu S. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med* 2009 **361** 1152-1163.

22. Gan W, Guan Y, Wu Q, An P, Zhu J, Lu L, Jing L, Yu Y, Ruan S, Xie D, Makrides M, Gibson RA, Anderson GJ, Li H, Lin X & Wang F. Association of TMPRSS6 polymorphisms with ferritin, hemoglobin, and type 2 diabetes risk in a Chinese Han population. *Am J Clin Nutr* 2012 **95** 626-632.

23. Herder C, Klopp N, Baumert J, Muller M, Khuseyinova N, Meisinger C, Martin S, Illig T, Koenig W & Thorand B. Effect of macrophage migration inhibitory factor (MIF) gene variants and MIF serum concentrations on the risk of type 2 diabetes: results from the MONICA/KORA Augsburg Case-Cohort Study, 1984-2002. *Diabetologia* 2008 **51** 276-284.

24. Huang T, Ren J, Huang J & Li D. Association of homocysteine with type 2 diabetes: a meta-analysis implementing Mendelian randomization approach. *BMC Genomics* 2013 **14** 867.

25. Huang T, Sun J, Chen Y, Xie H, Xu D & Li D. Associations of common variants in methionine metabolism pathway genes with plasma homocysteine and the risk of type 2 diabetes in Han Chinese. *J Nutrigenet Nutrigenomics* 2014 **7** 63-74.

26. Interleukin 1 Genetics C. Cardiometabolic effects of genetic upregulation of the interleukin 1 receptor antagonist: a Mendelian randomisation analysis. *Lancet Diabetes Endocrinol* 2015 **3** 243-253.

27. Jensen MK, Bartz TM, Djousse L, Kizer JR, Zieman SJ, Rimm EB, Siscovick DS, Psaty BM, Ix JH & Mukamal KJ. Genetically elevated fetuin-A levels, fasting glucose levels, and risk of type 2 diabetes: the cardiovascular health study. *Diabetes Care* 2013 **36** 3121-3127.

28. Jorde R, Schirmer H, Wilsgaard T, Joakimsen RM, Mathiesen EB, Njolstad I, Lochen ML, Figenschau Y, Berg JP, Svartberg J & Grimnes G. Polymorphisms related to the serum 25-

hydroxyvitamin D level and risk of myocardial infarction, diabetes, cancer and mortality. The Tromso Study. *PLoS One* 2012 **7** e37295.

29. Kamstrup PR & Nordestgaard BG. Lipoprotein(a) concentrations, isoform size, and risk of type 2 diabetes: a Mendelian randomisation study. *Lancet Diabetes Endocrinol* 2013 **1** 220-227.

30. Kroger J, Zietemann V, Enzenbach C, Weikert C, Jansen EH, Doring F, Joost HG, Boeing H & Schulze MB. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Am J Clin Nutr* 2011 **93** 127-142.

31. Leong A, Rehman W, Dastani Z, Greenwood C, Timpson N, Langsetmo L, Berger C, Metastroke, Fu L, Wong BY, Malik S, Malik R, Hanley DA, Cole DE, Goltzman D & Richards JB. The causal effect of vitamin D binding protein (DBP) levels on calcemic and cardiometabolic diseases: a Mendelian randomization study. *PLoS Med* 2014 **11** e1001751.

32. Magnusson M, Wang TJ, Clish C, Engstrom G, Nilsson P, Gerszten RE & Melander O. Dimethylglycine Deficiency and the Development of Diabetes mellitus. *Diabetes* 2015.

33. Perry JR, Ferrucci L, Bandinelli S, Guralnik J, Semba RD, Rice N, Melzer D, Consortium D, Saxena R, Scott LJ, McCarthy MI, Hattersley AT, Zeggini E, Weedon MN & Frayling TM. Circulating beta-carotene levels and type 2 diabetes-cause or effect? *Diabetologia* 2009 **52** 2117-2121.

34. Perry JR, Weedon MN, Langenberg C, Jackson AU, Lyssenko V, Sparso T, Thorleifsson G, Grallert H, Ferrucci L, Maggio M, Paolisso G, Walker M, Palmer CN, Payne F, Young E, Herder C, Narisu N, Morken MA, Bonnycastle LL, Owen KR, Shields B, Knight B, Bennett A, Groves CJ, Ruokonen A, Jarvelin MR, Pearson E, Pascoe L, Ferrannini E, Bornstein SR, Stringham HM, Scott LJ, Kuusisto J, Nilsson P, Neptin M, Gjesing AP, Pisinger C, Lauritzen T, Sandbaek A, Sampson M, Magic, Zeggini E, Lindgren CM, Steinthorsdottir V, Thorsteinsdottir U, Hansen T, Schwarz P, Illig T, Laakso M, Stefansson K, Morris AD, Groop L, Pedersen O, Boehnke M, Barroso I, Wareham NJ, Hattersley AT, McCarthy MI & Frayling TM. Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Hum Mol Genet* 2010 **19** 535-544.

35. Peters KE, Beilby J, Cadby G, Warrington NM, Bruce DG, Davis WA, Davis TM, Wiltshire S, Knuiman M, McQuillan BM, Palmer LJ, Thompson PL & Hung J. A comprehensive investigation of variants in genes encoding adiponectin (ADIPOQ) and its receptors (ADIPOR1/R2), and their association with serum adiponectin, type 2 diabetes, insulin resistance and the metabolic syndrome. *BMC Med Genet* 2013 **14** 15.

36. Pfister R, Barnes D, Luben R, Forouhi NG, Bochud M, Khaw KT, Wareham NJ & Langenberg C. No evidence for a causal link between uric acid and type 2 diabetes: a Mendelian randomisation approach. *Diabetologia* 2011 **54** 2561-2569.

37. Pfister R, Sharp S, Luben R, Welsh P, Barroso I, Salomaa V, Meirhaeghe A, Khaw KT, Sattar N, Langenberg C & Wareham NJ. Mendelian randomization study of B-type natriuretic peptide and type 2 diabetes: evidence of causal association from population studies. *PLoS Med* 2011 **8** e1001112.

38. Song Y, Yeung E, Liu A, Vanderweele TJ, Chen L, Lu C, Liu C, Schisterman EF, Ning Y & Zhang C. Pancreatic beta-cell function and type 2 diabetes risk: quantify the causal effect using a Mendelian randomization approach based on meta-analyses. *Hum Mol Genet* 2012 **21** 5010-5018.

39. Strawbridge RJ, Dupuis J, Prokopenko I, Barker A, Ahlqvist E, Rybin D, Petrie JR, Travers ME, Bouatia-Naji N, Dimas AS, Nica A, Wheeler E, Chen H, Voight BF, Taneera J, Kanoni S, Peden JF, Turrini F, Gustafsson S, Zabena C, Almgren P, Barker DJ, Barnes D, Dennison EM, Eriksson JG, Eriksson P, Eury E, Folkersen L, Fox CS, Frayling TM, Goel A, Gu HF, Horikoshi M, Isomaa B, Jackson AU, Jameson KA, Kajantie E, Kerr-Conte J, Kuulasmaa T, Kuusisto J, Loos RJ, Luan J, Makrilakis K, Manning AK, Martinez-Larrad MT, Narisu N, Nastase Mannila M, Ohrvik J, Osmond C, Pascoe L, Payne F, Sayer AA, Sennblad B, Silveira A, Stancakova A, Stirrups K, Swift AJ, Syvanen AC, Tuomi T, van 't Hooft FM, Walker M, Weedon MN, Xie W, Zethelius B, Consortium D, Consortium G, Mu TC, Consortium CA, Consortium CD, Ongen H, Malarstig A, Hopewell JC, Saleheen D, Chambers J, Parish S, Danesh J, Kooner J, Ostenson CG, Lind L, Cooper CC, Serrano-Rios M, Ferrannini E, Forsen TJ, Clarke R, Franzosi MG, Seedorf U, Watkins H, Froguel P, Johnson P, Deloukas P, Collins FS, Laakso M, Dermitzakis ET, Boehnke M, McCarthy MI, Wareham NJ, Groop L, Pattou F, Gloyn AL, Dedoussis GV, Lyssenko V, Meigs JB, Barroso I, Watanabe RM, Ingelsson E, Langenberg C, Hamsten A & Florez JC. Genome-wide association identifies nine common

variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. *Diabetes* 2011 **60** 2624-2634.
40. Trombetta M, Bonetti S, Boselli ML, Miccoli R, Trabetti E, Malerba G, Pignatti PF, Bonora E, Del Prato S & Bonadonna RC. PPARG2 Pro12Ala and ADAMTS9 rs4607103 as "insulin resistance loci" and "insulin secretion loci" in Italian individuals. The GENFIEV study and the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) 4. *Acta Diabetol* 2013 **50** 401-408.
41. Wang TT, Chen YJ, Sun LL, Zhang SJ, Zhou ZY & Qiao H. Affection of single-nucleotide polymorphisms in miR-27a, miR-124a, and miR-146a on susceptibility to type 2 diabetes mellitus in Chinese Han people. *Chin Med J (Engl)* 2015 **128** 533-539.
42. Yaghootkar H, Lamina C, Scott RA, Dastani Z, Hivert MF, Warren LL, Stancakova A, Buxbaum SG, Lyytikainen LP, Henneman P, Wu Y, Cheung CY, Pankow JS, Jackson AU, Gustafsson S, Zhao JH, Ballantyne CM, Xie W, Bergman RN, Boehnke M, el Bouazzaoui F, Collins FS, Dunn SH, Dupuis J, Forouhi NG, Gillson C, Hattersley AT, Hong J, Kahonen M, Kuusisto J, Kedenko L, Kronenberg F, Doria A, Assimes TL, Ferrannini E, Hansen T, Hao K, Haring H, Knowles JW, Lindgren CM, Nolan JJ, Paananen J, Pedersen O, Quertermous T, Smith U, Consortium G, Consortium R, Lehtimaki T, Liu CT, Loos RJ, McCarthy MI, Morris AD, Vasan RS, Spector TD, Teslovich TM, Tuomilehto J, van Dijk KW, Viikari JS, Zhu N, Langenberg C, Ingelsson E, Semple RK, Sinaiko AR, Palmer CN, Walker M, Lam KS, Paulweber B, Mohlke KL, van Duijn C, Raitakari OT, Bidulescu A, Wareham NJ, Laakso M, Waterworth DM, Lawlor DA, Meigs JB, Richards JB & Frayling TM. Mendelian randomization studies do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. *Diabetes* 2013 **62** 3589-3598.
43. Yaghootkar H, Scott RA, White CC, Zhang W, Speliotes E, Munroe PB, Ehret GB, Bis JC, Fox CS, Walker M, Borecki IB, Knowles JW, Yerges-Armstrong L, Ohlsson C, Perry JR, Chambers JC, Kooner JS, Franceschini N, Langenberg C, Hivert MF, Dastani Z, Richards JB, Semple RK & Frayling TM. Genetic evidence for a normal-weight "metabolically obese" phenotype linking insulin resistance, hypertension, coronary artery disease, and type 2 diabetes. *Diabetes* 2014 **63** 4369-4377.
44. Ye Z, Haycock PC, Gurdasani D, Pomilla C, Boekholdt SM, Tsimikas S, Khaw KT, Wareham NJ, Sandhu MS & Forouhi NG. The association between circulating lipoprotein(a) and type 2 diabetes: is it causal? *Diabetes* 2014 **63** 332-342.
45. Ye Z, Sharp SJ, Burgess S, Scott RA, Imamura F, InterAct C, Langenberg C, Wareham NJ & Forouhi NG. Association between circulating 25-hydroxyvitamin D and incident type 2 diabetes: a mendelian randomisation study. *Lancet Diabetes Endocrinol* 2015 **3** 35-42.
46. You NC, Chen BH, Song Y, Lu X, Chen Y, Manson JE, Kang M, Howard BV, Margolis KL, Curb JD, Phillips LS, Stefanick ML, Tinker LF & Liu S. A prospective study of leukocyte telomere length and risk of type 2 diabetes in postmenopausal women. *Diabetes* 2012 **61** 2998-3004.
47. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, Zeggini E, Huth C, Aulchenko YS, Thorleifsson G, McCulloch LJ, Ferreira T, Grallert H, Amin N, Wu G, Willer CJ, Raychaudhuri S, McCarroll SA, Langenberg C, Hofmann OM, Dupuis J, Qi L, Segre AV, van Hoek M, Navarro P, Ardlie K, Balkau B, Benediktsson R, Bennett AJ, Blagieva R, Boerwinkle E, Bonnycastle LL, Bengtsson Bostrom K, Bravenboer B, Bumpstead S, Burtt NP, Charpentier G, Chines PS, Cornelis M, Couper DJ, Crawford G, Doney AS, Elliott KS, Elliott AL, Erdos MR, Fox CS, Franklin CS, Ganser M, Gieger C, Grarup N, Green T, Griffin S, Groves CJ, Guiducci C, Hadjadj S, Hassanali N, Herder C, Isomaa B, Jackson AU, Johnson PR, Jorgensen T, Kao WH, Klopp N, Kong A, Kraft P, Kuusisto J, Lauritzen T, Li M, Lieverse A, Lindgren CM, Lyssenko V, Marre M, Meitinger T, Midthjell K, Morken MA, Narisu N, Nilsson P, Owen KR, Payne F, Perry JR, Petersen AK, Platou C, Proenca C, Prokopenko I, Rathmann W, Rayner NW, Robertson NR, Rocheleau G, Roden M, Sampson MJ, Saxena R, Shields BM, Shrader P, Sigurdsson G, Sparso T, Strassburger K, Stringham HM, Sun Q, Swift AJ, Thorand B, Tichet J, Tuomi T, van Dam RM, van Haeften TW, van Herpt T, van Vliet-Ostaptchouk JV, Walters GB, Weedon MN, Wijmenga C, Witteman J, Bergman RN, Cauchi S, Collins FS, Gloyn AL, Gyllensten U, Hansen T, Hide WA, Hitman GA, Hofman A, Hunter DJ, Hveem K, Laakso M, Mohlke KL, Morris AD, Palmer CN, Pramstaller PP, Rudan I, Sijbrands E, Stein LD, Tuomilehto J, Uitterlinden A, Walker M, Wareham NJ, Watanabe RM, Abecasis GR, Boehm BO, Campbell H, Daly MJ, Hattersley AT, Hu FB, Meigs JB, Pankow JS, Pedersen O, Wichmann HE, Barroso I, Florez JC, Frayling TM, Groop L, Sladek R, Thorsteinsdottir U, Wilson JF, Illig T, Froguel P, van Duijn CM, Stefansson K, Altshuler D, Boehnke M, McCarthy MI, investigators M & Consortium G. Twelve type 2 diabetes

susceptibility loci identified through large-scale association analysis. *Nat Genet* 2010 **42** 579-589.

48. Van Campenhout A, Van Campenhout C, Lagrou AR, Abrams P, Moorkens G, Van Gaal L & Manuel-y-Keenoy B. Impact of diabetes mellitus on the relationships between iron-, inflammatory- and oxidative stress status. *Diabetes Metab Res Rev* 2006 **22** 444-454.
49. Sedlak TW & Snyder SH. Messenger molecules and cell death: therapeutic implications. *JAMA* 2006 **295** 81-89.
50. Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, Yamahara K, Taura D, Inuzuka M, Sonoyama T & Nakao K. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. *Diabetes* 2009 **58** 2880-2892.
51. Corpeleijn E, Feskens EJ, Jansen EH, Mensink M, Saris WH, de Bruin TW & Blaak EE. Improvements in glucose tolerance and insulin sensitivity after lifestyle intervention are related to changes in serum fatty acid profile and desaturase activities: the SLIM study. *Diabetologia* 2006 **49** 2392-2401.
52. Vessby B, Gustafsson IB, Tengblad S, Boberg M & Andersson A. Desaturation and elongation of Fatty acids and insulin action. *Ann N Y Acad Sci* 2002 **967** 183-195.
53. Rhee EP, Ho JE, Chen MH, Shen D, Cheng S, Larson MG, Ghorbani A, Shi X, Helenius IT, O'Donnell CJ, Souza AL, Deik A, Pierce KA, Bullock K, Walford GA, Vasan RS, Florez JC, Clish C, Yeh JR, Wang TJ & Gerszten RE. A genome-wide association study of the human metabolome in a community-based cohort. *Cell Metab* 2013 **18** 130-143.
54. Konstantinova SV, Tell GS, Vollset SE, Nygard O, Bleie O & Ueland PM. Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. *J Nutr* 2008 **138** 914-920.
55. Ho JE, Larson MG, Vasan RS, Ghorbani A, Cheng S, Rhee EP, Florez JC, Clish CB, Gerszten RE & Wang TJ. Metabolite profiles during oral glucose challenge. *Diabetes* 2013 **62** 2689-2698.
56. Sheikh K, Camejo G, Lanne B, Halvarsson T, Landergren MR & Oakes ND. Beyond lipids, pharmacological PPARalpha activation has important effects on amino acid metabolism as studied in the rat. *Am J Physiol Endocrinol Metab* 2007 **292** E1157-1165.
57. Ahluwalia TS, Allin KH, Sandholt CH, Sparso TH, Jorgensen ME, Rowe M, Christensen C, Brandslund I, Lauritzen T, Linneberg A, Husemoen LL, Jorgensen T, Hansen T, Grarup N & Pedersen O. Discovery of coding genetic variants influencing diabetes-related serum biomarkers and their impact on risk of type 2 diabetes. *J Clin Endocrinol Metab* 2015 **100** E664-671.
58. Kraja AT, Chasman DI, North KE, Reiner AP, Yanek LR, Kilpelainen TO, Smith JA, Dehghan A, Dupuis J, Johnson AD, Feitosa MF, Tekola-Ayele F, Chu AY, Nolte IM, Dastani Z, Morris A, Pendergrass SA, Sun YV, Ritchie MD, Vaez A, Lin H, Ligthart S, Marullo L, Rohde R, Shao Y, Ziegler MA, Im HK, Cross Consortia Pleiotropy G, Cohorts for Heart a, Aging Research in Genetic E, Genetic Investigation of Anthropometric Traits C, Global Lipids Genetics C, Meta-Analyses of G, Insulin-related traits C, Global BC, Consortium AD, Women's Genome Health S, Howard University Family S, Schnabel RB, Jorgensen T, Jorgensen ME, Hansen T, Pedersen O, Stolk RP, Snieder H, Hofman A, Uitterlinden AG, Franco OH, Ikram MA, Richards JB, Rotimi C, Wilson JG, Lange L, Ganesh SK, Nalls M, Rasmussen-Torvik LJ, Pankow JS, Coresh J, Tang W, Linda Kao WH, Boerwinkle E, Morrison AC, Ridker PM, Becker DM, Rotter JI, Kardia SL, Loos RJ, Larson MG, Hsu YH, Province MA, Tracy R, Voight BF, Vaidya D, O'Donnell CJ, Benjamin EJ, Alizadeh BZ, Prokopenko I, Meigs JB & Borecki IB. Pleiotropic genes for metabolic syndrome and inflammation. *Mol Genet Metab* 2014 **112** 317-338.
59. Vaez A, Jansen R, Prins BP, Hottenga JJ, de Geus EJ, Boomsma DI, Penninx BW, Nolte IM, Snieder H & Alizadeh BZ. In Silico Post Genome-Wide Association Studies Analysis of C-Reactive Protein Loci Suggests an Important Role for Interferons. *Circ Cardiovasc Genet* 2015 **8** 487-497.

Table 1: Mendelian randomization studies for biomarkers and type 2 diabetes

Biomarkers	No. of Study	Study	Country/year	Cases/ total or controls	Genetic variant(s)/locus	Causal estimate	Type of associations
Adiponectin	3[19, 35, 42]	DIAGRAMv2, ARIC, FUSION, WTCCC, EPIC-InterAct	US, Europe/2013	15960/64731	rs17300539,rs1736653,rs3774261,rs3821799/*ADIPOQ*	Null	
		BHS, CUDAS, FDS	Australia/2013	967/2355	rs12637534, rs16861209, rs17366568/*ADIPOQ*	OR,0.86	per allele
		DIAGRAMv2	US/Europe/2012	-/22044	rs3001032/*LYPLAL1*, rs1108842/*GNL3*, rs1597466/*TSC22D2*, rs6810075/*ADIPOQ*, rs998584/*VEGFA*, rs2980879/*TRIB1*, rs7955516/*PDE3A*, rs601339/*GPR109A*, rs7133378/*DNAH10*, rs2925979/*CMIP*, rs12922394/*CDH13*, rs731839/*PEPD*	β, -0.30	average additive effect of adiponectin-raising alleles
Beta-carotene	1[33]	WTCCC, DGI, FUSION	US, Europe/2009	4549/5579	rs6564851/*BCMO1*	Null	
Bilirubin	1[11]	PREVEND	Netherlands/ 2015	210/3381	rs6742078/*UGT1A1*	OR, 0.58	1-sd
B-type proBNP	1[37]	DIAGRAMv2, Norfolk Diabetes, Cambridgeshire, ADDITION-Ely, French, Swiss 1 , Swiss 2, Austrian, MONICA, INCA, UK	US, Europe/2011	23382/57898	rs198389/*NPPB*	OR, 0.96	*C* allele
C-reactive protein	1[16]	Whitehall II	UK/2008	354/ 5274	rs1130864, rs1205, rs3093077/ *CRP*	Null	
Delta-6 desaturase	1[30]	EPIC-Potsdam	Germany/2011	673/2724	rs174546/*FADS1*	RR, 0.64	*TT* genotype
Dimethylglycine	1[32]	MDC-CC, MDC, MPP	Sweden/2015	4201/33898	rs2431332/ *DMGDH*	OR, 1.1	*A* allele
Ferritin/TMPRSS6	1[22]	Beijing	China/2012	272/1574	rs855791, rs4820268/ *TMPRSS6*	OR, 0.79	*AG* haplotype
Fetuin-a	1[27]	CHS	US/2013	259/3093	rs4917, rs2248690/*AHSG*	Null	
Homocysteine	2[24, 25]	Hangzhou	China/2014	774/500	rs1801131/*MTHFR*, rs4646356/*PEMT*	OR,1.93, 1.52	*CC*, *CT+TT*
		-	Europe,Asia, Africa/2013	4011/ 4303	rs12134663, rs1801133/*MTHFR*	OR,1.29	5μmol/L
IL-1Ra	1[26]	DIAGRAMv3 and EPIC-InterAct	US, Europe/2015	18715/61692	rs6743376, rs1542176/*IL1RN*	Null	

Leukocytr telomere length	1[46]	WHI-OS	US/2012	1675/2382	rs34368910/*TRF1*, rs4888444/*TRF2*, rs4975605/*TERT*, rs938886, rs2228041,rs12880583/*TPP1*	Null	
Lipoprotein(a)	2[29, 44]	Danish general population	Denmark/2014	2157/28567	rs10455872/*LPA*	Null	
		DIAGRAMv3,EPIC-Norfolk	US, Europe/2014	10088/68346	rs10455872/*LPA*	Null	
Macrophage migration inhibitory factor	1[23]	MONICA/KORA Augsburg	Germany/2008	502/1632	rs1007888/*MIF*	HR, 1.74	*C* allele
miRNAs	1[41]	Harbin	China/2015	995/967	rs895819/miR-27a, rs531564/miR-124a, rs2910164/miR-146a	Null	
Resistin	1[18]	CVDFACTS	Taiwan/2014	230/3400	rs3745367,rs1423096/*RETN*	OR, 1.38	*GG*
Sex hormone binding globulin	2[21, 34]	WTCCC, Dundee, EFS-Y2D, Danish, DGI, MCC, FUSION 1-2, KORA, Cambridgeshire, ADDITION-Ely, NDCCS, DeCODE, METSIM, DIAGEN		27657/58481	rs1799941/*SHBG*	OR, 0.92	*G* allele
		WHS	US/2009	359/359	rs6259/*SHBG*	OR, 0.3	1-sd
Triglycerides	1[20]	Go-DARTS	UK/2011	5637/ 6860	rs2954029/ *TRIB1*, rs714052/*MLXIPL*, rs7557067/*APOB*, rs17216525/*NCAN*, rs10889353/*ANGPTL3*, rs7679/*PLTP*, rs7819412/*XKR6-AMAC1L2*, rs328/*LPL*, rs3135506/*APOA5*, rs662799/*APOA5*	Null	
Uric acid	1[36]	Cambridgeshire, ADDITION-Ely, Norfolk Diabetes	UK/2011	7504/ 8560	rs12129861/ *PDZK1,* rs742132/*LRRC16A*, rs505802/*SLC22A12*, rs12356193/ *SLC16A9*, rs17300741/ *SLC22A11*, rs1165151/ *SLC17A1*, rs2231142/ *ABCG2,* rs734553/ *SLC2A9*	Null	
Vitamin D	3[17, 28, 45]	DIAGRAMv3, ADDITION-Ely, Norfolk Diabetes, Cambridgeshire, EPIC-InterAct	US, Europe/2015	28144/76344	rs12785878/DHCR7,rs10741657/CYP2R1,rs4588/DBP,rs17217119/CYP24A1	Null	
		EPIC-Potsdam	Germany/2013	1572/2121	rs2282679,rs1155563,rs12785878,rs3829251,rs10741657,rs6013897,rs6599638, rs10877012/*DBP GC*, *DHCR7*,*CYP2R1*,*CYP24A1*,Ch10orf88,*CYP27B1*	Null	

		Tromsø	Norway/2012	1092/9528	rs2298850/*DBP GC*, rs10741657/CYP2R1, rs3794060/NADSYN1, rs6013897/*CYP24A1*	Null	
Vitamin D binding protein	1[31]	CaMos	Canada/2014	201/2254	rs2282679/*DBP GC*	Null	

OR, odds ratio; RR, relative risk; HR, hazard ratio; BNP, brain natriuretic peptide, interleukin 1 receptor antagonist (IL1-Ra)

DIAGRAM, DIAbetes Genetics Replication And Meta-analysis; EPIC-InterAct, European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct; ARIC, Atherosclerosis Risk in Communities; WTCCC, Welcome Trust Case Control Cohort; FUSION, Finland-US Investigation of NIDDM genetics

BHS, Busselton Population Health Survey; CUDAS, Carotid Ultrasound Disease Assessment Study; FDS, Fremantle Diabetes Study

PREVEND, Prevention of Renal and Vascular End-stage Disease;

iNCA, iNsuffisance CAdiaque; MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease

MDC, Malmö Diet and Cancer Study; MDC-CC, Malmö Diet and Cancer Cardiovascular Cohort; MPP, Malmö Preventive Project;
CVDFACTS , CardioVascular Disease risk FACtors Two-township Study

KORA, kooperative gesundheitsforschung in der region Augsburg; EFS-Y2D; DGI, Diabetes Genetics Initiative; Norfolk Diabetes Case Control Study, NDCCS; METSIM, Metabolic Syndrome in Men; DIAGEN, DIAbetes GENetic Study

CHS, Cardiovascular Health Study

WHI-OS, Women's Health Initiative Observational Study Cohort

ADDITION, Anglo-Danish-Dutch Study of Intensive Treatment In People with Screen Detected Diabetes in Primary Care

MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease

Genetics of Diabetes Audit and Research in Tayside Scotland, Go-DARTS
CaMos, Canadian Multicentre Osteoporosis Study

Table 2: Nominally significance for each biomarker-associated genetic variants with glucose, HOMA-IR and the risk of type 2 diabetes

		P value*			
Biomarkers	**Genetic variants**	**T2DM[13]**	**Glucose[14]**	**HOMA-IR[14]**	**Nominally significance**
Adiponectin	rs1736653	-	-	-	NA
	rs2980879	0.96	0.9613	0.9276	Null
	rs16861209	0.89	0.846	0.505	Null
	rs601339	0.89	0.8344	0.02762	Yes
	rs17300539	0.87	0.8116	0.1489	Null
	rs7955516	0.86	0.8366	0.8286	Null
	rs12922394	0.54	0.07314	0.1932	Null
	rs3821799	0.51	0.1882	0.7989	Null
	rs731839	0.41	0.3386	0.01373	Yes
	rs3774261	0.32	0.4418	0.7559	Null
	rs17366568	0.31	0.9738	0.3994	Null
	rs7133378	0.16	0.2134	0.3286	Null
	rs12637534	0.15	0.379	0.0006	Yes
	rs6810075	0.1	0.7728	0.5248	Null
	rs998584	0.06	0.808	0.6827	Null
	rs1108842	0.032	0.4706	0.05447	Yes
	rs3001032	0.029	0.2725	0.0001	Yes
	rs1597466	0.0087	0.06167	0.4695	Yes
	rs2925979	0.0023	0.05063	0.1782	Yes
Beta-carotene	rs6564851	0.22	0.3938	0.2702	Null
Bilirubin	rs6742078	0.1	0.0239	0.03327	Yes
B-type proBNP	rs198389	0.034	0.3651	0.3891	Yes
CRP	rs1205	0.66	0.6836	0.6402	Null
	rs3093077	0.048	0.9494	0.393	Yes
	rs1130864	-	0.1199	0.7696	Null
D6-desaturase	rs174546	0.0032	2.7×10^{-8}	0.8911	Yes
Dimethylglycine	rs2431332	0.51	0.00421	0.2899	Yes
Ferritin/TMPRSS6	rs855791	1	0.09508	0.2457	Null
	rs4820268	0.73	0.1492	0.1271	Null
Fetuin-a	rs4917	0.082	0.1046	0.6666	Null
	rs2248690	0.055	0.9571	0.7713	Null
Homocysteine	rs4646356	0.88	0.4332	0.3475	Null
	rs12134663	0.78	0.6857	0.6192	Null
	rs1801131	0.22	0.6088	0.2367	Null
	rs1801133	0.096	0.459	0.4294	Null
IL-1Ra	rs1542176	0.64	0.00802	0.7087	Yes
	rs6743376	-	0.9109	0.4552	Null
LTL	rs12880583	-	-	-	NA
	rs2228041	-	-	-	NA
	rs34368910	-	-	-	NA
	rs938886	0.88	0.04159	0.7375	Yes
	rs4975605	0.66	0.4506	0.6104	Null
	rs4888444	0.086	0.9143	0.3436	Null
Lp(a)	rs10455872	0.36	0.4242	0.3231	Null
MIF	rs1007888	0.39	0.2167	0.9401	Null
miRNAs	rs12610873	0.033	0.8022	0.7117	Yes
	rs531564	0.66	0.5302	0.9714	Null
	rs2910164	0.52	0.01256	0.3573	Yes
Resistin	rs3745367	0.77	0.9917	0.5793	Null
	rs1423096	0.72	0.8155	0.3391	Null
SHBG	rs12150660	0.18	0.5165	0.2413	Null
	rs6259	0.94	0.4235	0.3504	Null
Triglycerides	rs12285095	0.41	0.9737	0.6178	Null
	rs7557067	0.9	0.7824	0.1467	Null

	rs7679	0.88	0.9	0.2909	Null
	rs2954029	0.72	0.6368	0.871	Null
	rs662799	0.28	0.04162	0.5552	Yes
	rs714052	0.28	0.807	0.8035	Null
	rs10889353	0.19	0.4164	0.2106	Null
	rs7819412	0.079	0.8028	0.00979	Yes
	rs328	0.025	0.05081	0.03923	Yes
	rs17216525	0.0035	0.9843	0.8103	Yes
Uric acid	rs1165151	0.74	0.4165	0.8597	Null
	rs2231142	0.73	0.8335	0.3608	Null
	rs505802	0.62	0.6034	0.6212	Null
	rs734553	0.57	0.1985	0.7163	Null
	rs742132	0.25	0.5028	0.9073	Null
	rs12356193	0.14	0.9486	0.4696	Null
	rs12129861	0.083	0.9366	0.502	Null
	rs17300741	0.026	0.3446	0.3608	Yes
Vitamin-D	rs10877012	-	-	-	NA
	rs3755967	0.74	0.8454	0.1899	Null
	rs1155563	0.93	0.7691	0.06121	Null
	rs2298850	0.66	0.8892	0.1472	Null
	rs3829251	0.54	0.08705	0.2639	Null
	rs3794060	0.34	0.2832	0.1035	Null
	rs6599638	0.3	0.1749	0.4272	Null
	rs10741657	0.23	0.3746	0.0788	Null
	rs12785878	0.14	0.3232	0.1677	Null
	rs17217119	0.061	0.3553	0.2098	Null
	rs6013897	0.057	0.3293	0.1185	Null
Vitamin D-BP	rs2282679	0.76	0.997	0.1191	Null

*p values for each of the biomarker variants were extracted from publicly available meta-analyses of genome-wide association studies [13, 14].

NA, not applicable; CRP, C-reactive protein; BNP, brain natriuretic peptide; interleukin 1 receptor antagonist (IL1-Ra); Lp(a)Lipoprotein(a); MIF, Macrophage migration inhibitory factor; LTL, Leukocyte telomere length; SHBG, Sex hormone binding globulin, Vitamin D-BP, Vitamin D binding protein