Article

Evaluating the impact of AMPK activation, a target of metformin, on risk of cardiovascular diseases and cancer in the UK Biobank: a Mendelian randomisation study

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Received: 27 March 2020 / Accepted: 17 June 2020

Abstract

Aims/hypothesis Whether metformin reduces cardiovascular or cancer risk is unclear owing to concerns over immortal time bias and confounding in observational studies. This study evaluated the effect of AMP-activated protein kinase (AMPK), the target of metformin, on risk of cardiovascular disease and cancer.

Methods This is a Mendelian randomisation design, using AMPK, the pharmacological target of metformin, to infer the AMPK pathway-dependent effects of metformin on risk of cardiovascular disease and cancer in participants of white British ancestry in the UK Biobank.

Results A total of 391,199 participants were included (mean age 56.9 years; 54.1% women), including 26,690 cases of type 2 diabetes, 38,098 cases of coronary artery disease and 80,941 cases of overall cancer. Genetically predicted reduction in HbA_{1c} (%) instrumented by AMPK variants was associated with a 61% reduction in risk of type 2 diabetes (OR 0.39; 95% CI 0.20, 0.78; $p=7.69\times10^{-3}$), a 53% decrease in the risk of coronary artery disease (OR 0.47; 95% CI 0.26, 0.84; $p=0.01$) and a 44% decrease in the risk of overall cancer (OR 0.56; 95% CI 0.36, 0.85; $p=7.23\times10^{-3}$). Results were similar using median or quartiles of AMPK score, with dose–response effects (p for trend=4.18×10^{-3} for type 2 diabetes, 4.37×10^{-3} for coronary artery disease and 4.04×10^{-3} for overall cancer).

Conclusions/interpretation This study provides some genetic evidence that AMPK activation by metformin may protect against cardiovascular disease and cancer, which needs to be confirmed by randomised controlled trials.

Keywords: AMPK, Cancer, Coronary artery disease, Mendelian randomisation, Metformin, Type 2 diabetes, UK Biobank

Abbreviations

AMPK	AMP-activated protein kinase
GDF-15	Growth differentiation factor 15
GWAS	Genome-wide association study
IFCC	International Federation of Clinical Chemistry
MAGIC	Meta-Analyses of Glucose and Insulin-related traits Consortium
NGSP	National Glycohemoglobin Standardization Program

Research in context

What is already known about this subject?

- Metformin is the first-line pharmacological treatment to manage hyperglycaemia in people with type 2 diabetes
- Whether metformin reduces cardiovascular or cancer risk is unclear due to concerns over immortal time bias and confounding in observational studies

What is the key question?

- What is the association of AMP-activated protein kinase (AMPK), the pharmacological target of metformin, with lifetime risk of cardiovascular disease and cancer?

What are the new findings?

- In Mendelian randomisation analyses involving 391,199 participants of white British ancestry in the UK Biobank, reduction in HbA_{1c} instrumented by AMPK was associated with lower risk of coronary artery disease, and possibly lower risk of cancer

How might this impact on clinical practice in the foreseeable future?

- Based on the genetics of AMPK, metformin use may protect against cardiovascular disease, and possibly cancer

Introduction

Metformin is the first-line pharmacological treatment to manage hyperglycaemia in people with type 2 diabetes, and is on the World Health Organization list of essential medicines [1]. Increasing evidence suggests that metformin may differ from other classes of glucose-lowering medications in having superior safety and lower risk of cardiovascular complications [2]. Furthermore, pharmaco-epidemiological studies have suggested that metformin may reduce cardiovascular disease and cancer [3, 4], suggesting the possibility of its use for these diseases. Metformin not only impacts glycaemic traits but also other potentially relevant factors, such as growth differentiation factor 15 (GDF-15) and vascular endothelial growth factors [5]. However, pharmaco-epidemiological studies may be open to immortal time bias and confounding, which may generate spurious protective effects of metformin, in particular for cancer-related studies [6, 7]. To date, relevant randomised controlled trials of metformin in cardiovascular disease are not large enough to be definitive [8], whilst the impact of metformin on cancer has not been evaluated fully in a randomised controlled trial.

Mendelian randomisation studies, which make use of the random allocation of genetic variants at conception, are less susceptible to confounding and time-related biases than other observational studies, and are now increasingly used to infer health effects of medications, such as the use 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*HMGCR*) variants to mimic the health effects of statins [9]. Previous Mendelian randomisation studies have attempted to use genetics to infer health effects of metformin, but they were potentially underpowered and included non-specific instruments [10], or only evaluated health effects of metformin biomarkers instead of metformin itself [11]. To provide more definitive and direct evidence concerning the effects of metformin on cardiovascular disease and cancer risk, we

conducted a Mendelian randomisation study using AMP-activated protein kinase (AMPK), the target of metformin, as a proxy of metformin use, in one of the largest prospective cohort studies globally.

Methods

Study design This is a Mendelian randomisation design, using AMPK, the pharmacological target of metformin, to infer the AMPK pathway-dependent effects of metformin. The study design is depicted in Fig. 1 [12].

Study population The UK Biobank recruited ~500,000 participants intended to be aged 39-73 years between 2006 and 2010 from 22 recruitment centres across Scotland, Wales and England in the UK. Participants provided biological samples; completed questionnaires, covering self-reported diseases and regular prescription medications; underwent assessments; and had nurse-led interviews. A blood sample for standard haematological tests was collected by venepuncture in ethylenediaminetetraacetic acid tubes, and tested at the central processing laboratory in Stockport, within 24 h of blood collection. HbA_{1c} was measured by high performance liquid chromatography on Bio-Rad Variant II Turbo analysers (Bio-Rad Laboratories, California). Longitudinal follow-up via record linkage to all health service encounters and death is ongoing. Hospital inpatient data and cancer registries used ICD-9 and ICD-10 codes and death registries used ICD-10 codes. Genotyping was undertaken with two similar arrays, the UK Biobank Lung Exome Variant Evaluation (BiLEVE) Axiom array (49,979 participants) and the UK Biobank Axiom array (438,398 participants). Genotype imputation was based on the reference panel combining the UK10K haplotype and the Haplotype Reference Consortium reference panels. To reduce confounding by latent population structure [13], we restricted the analysis to genetically verified white British participants and further excluded participants with (1) withdrawn consent; (2) sex mismatch

(genetic sex differs from reported sex); (3) putative sex chromosomes aneuploidy; (4) poor-quality genotyping (outliers in heterozygosity and missing rate >1.5%); or (5) excessive relatedness (more than ten putative third-degree relatives), as per our previous study [14]. We used genotype and phenotype data from the UK Biobank provided in February 2020.

AMPK genetic score We created a weighted AMPK genetic score to mimic the effects of AMPK activation by metformin use based on the strength of the association of genetic variants in the relevant gene regions with HbA_{1c} in the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC), a genome-wide association study (GWAS) of HbA_{1c}, with validation in the UK Biobank. Specifically, we selected genetic variants within 1 megabase pairs downstream and upstream of each of the *PRKAA1*, *PRKAA2*, *PRKAB1*, *PRKAB2*, *PRKAG1*, *PRKAG2* and *PRKAG3* genes that encode AMPK subunits [15]. We selected low-linkage disequilibrium ($r^2<0.3$) variants associated with HbA_{1c} at a nominal level of statistical significance ($p\leq0.05$) in MAGIC, restricted to people of European ancestry to minimise population stratification (n=123,665) [16]. We then validated the associations in the UK Biobank (using multivariable linear regression, adjusted for age, sex, age at recruitment, genotyping array and the first 20 principal components of genetic ancestry) and only retained variants also reaching statistical significance ($p\leq0.05$) in the UK Biobank, which were used to construct the AMPK score. Electronic supplementary material (ESM) Table 1 and ESM Fig. 1 show the details regarding the 44 variants used to construct the AMPK score. A weighted AMPK score was calculated for each participant by summing the number of HbA_{1c}-lowering alleles that a participant inherited at each variant included in the AMPK score, weighted by the effect of that variant on HbA_{1c} measured in percentage (as estimated in MAGIC) [16]. This score was then considered as above and below the median to mimic metformin use and non-use. We also considered AMPK score in quartiles to assess whether our finding was robust to the way we consider AMPK scores.

Sensitivity analysis As a sensitivity analysis, we used a stringent variant selection criterion by using only variants associated with HbA_{1c} at genome-wide significance ($p \leq 5 \times 10^{-8}$) in both MAGIC and UK Biobank and not in linkage disequilibrium with the other variants ($r^2 < 0.01$), which gave rs2732480 (ESM Table 1). rs2732480 was associated with lower HbA_{1c} in both MAGIC ($p = 2 \times 10^{-9}$) and UK Biobank ($p = 1.07 \times 10^{-142}$). The effect allele was associated with lower HbA_{1c} percentage (β -0.012; 95% CI -0.016, -0.008).

Study outcomes The primary outcomes were coronary artery disease and overall cancer. The secondary outcomes were stroke and three main cancers, i.e. breast cancer, colorectal cancer and prostate cancer. Each disease outcome was defined based on self-reported medical conditions at baseline, or subsequent primary and secondary diagnoses of hospital episodes (ICD-9 and ICD-10), or cancer diagnosis (ICD-9 and ICD-10), or underlying and contributing causes of death (ICD-10).

Positive control outcomes We included type 2 diabetes and HbA_{1c} as positive control outcomes given that these are the expected effects of metformin use. Type 2 diabetes was ascertained using a validated algorithm [17]. Specifically, the criteria included (1) self-reported type 2 diabetes at baseline; (2) indication of type 2 diabetes based on diagnostic codes (ICD-9 250 and ICD-10 E11); (3) diabetes medications (metformin, sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide 1 receptor agonists, dipeptidyl peptidase 4 inhibitors, sulfonylureas and thiazolidinediones); and (4) hyperglycaemic blood result (either $HbA_{1c} \geq 6.5\%$ or 48 mmol/mol, or random glucose ≥ 11.1 mmol/l). The algorithmic definitions are described in ESM Table 2. HbA_{1c} was measured in mmol/mol (International Federation of Clinical Chemistry [IFCC] unit), and was converted to percentage (National Glycohemoglobin Standardization Program [NGSP] unit) using the equation: NGSP=(0.09148×IFCC)+2.152 [18].

External validation To validate our findings from the UK Biobank, we conducted an external validation study for the outcomes using summary statistics for type 2 diabetes (12,171 cases and 56,862 control participants) from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) consortium [19], for coronary artery disease (60,801 cases and 123,504 control participants) from the Coronary ARtery DIsease Genome wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics (CARDIoGRAMplusC4D) consortium [20], for stroke (40,585 cases of stroke and 406,111 control participants) from the MEGASTROKE consortium [21], for breast cancer (122,977 cases and 105,974 control participants) from the Breast Cancer Association Consortium (BCAC) [22] and for prostate cancer (79,148 cases and 61,106 control participants) from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium [23]. All participants were of predominantly European ancestry and non-overlapping with the participants in the UK Biobank to avoid bias due to population structure and any potential bias due to participant overlap for a weak instrument [24].

Statistical analysis To assess the assumption of independence of the genetic instruments (AMPK groups) from potential confounders, we assessed the association of AMPK groups with confounders (age at recruitment, BMI, smoking status, alcohol drinking status, education level, Townsend deprivation index) using χ^2 tests or ANOVA. To demonstrate that AMPK had the expected effect on HbA_{1c}, we assessed the differences in HbA_{1c} and random glucose between each group using ANOVA. We assessed the association of AMPK categories with HbA_{1c} using multivariable linear regression. We assessed the association of AMPK categories with risk of type 2 diabetes, cardiovascular diseases and cancers using multivariable logistic regression. All regression analyses were adjusted for sex (if relevant), age at recruitment, genotyping array and the first 20 principal components of genetic ancestry. As per previous studies, we also assessed the impact of genetically predicted

reduction in HbA_{1c} (%) instrumented by AMPK variants on risk of type 2 diabetes, coronary artery disease and overall cancer [25].

For the external validation, we performed a standard Mendelian randomisation analysis. We obtained the summary statistics of each variant included in the AMPK score on risk of type 2 diabetes, coronary artery disease, stroke, breast cancer and prostate cancer as reported by each consortium. We obtained the Wald ratio for each variant (the ratio of the genetic association with outcome to the genetic association of exposure), and then combined them using weighted generalised linear regression in an inverse variance-weighted manner and accounted for the correlation between variants [26]. The correlations between variants were obtained in 503 participants of European ancestry from the 1000 Genomes Project (Phase 3). Since variants are from multiple gene regions that may have different mechanisms of effect, a random-effects model was used [26]. We aligned the effect allele of each variant to the HbA_{1c} decreasing allele. We used the Cochran's *Q* statistic to assess heterogeneity of the Wald ratios [27], where high heterogeneity may indicate the presence of invalid genetic variants [28].

Exploring the association of HbA_{1c} with cardiovascular disease and cancer risk using Mendelian randomisation To preclude the possibility that the observed effects of AMPK activation, a target of metformin, are due to lowering HbA_{1c}, we also assessed the association of genetically predicted lower HbA_{1c} on cardiovascular disease and cancer risk. As previously, we obtained 38 independent genetic variants strongly related to HbA_{1c} ($p \leq 5 \times 10^{-8}$) from MAGIC (ESM Table 3), and applied them to the relevant outcomes in the UK Biobank using inverse variance weighting, MR-Egger and a weighted median method [14].

The AMPK score was generated using *PLINK 2.0* (https://www.cog-genomics.org/plink/2.0/

).[29] Mendelian randomisation analyses were performed with the *MendelianRandomisation* package version 0.4.2 [30] and all analyses were performed using R software, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) [31]. A two-tailed *p* value less than 0.05 was considered statistically significant.

Ethical approval The UK Biobank received ethical approval from the North West Multi-centre Research Ethics Committee (11/NW/0382), and all participants provided written, informed consent. No ethics approval was acquired for the analyses using summary statistics. The contributing studies to the consortium received ethical approval from their specific institutional review boards, and written, informed consent was obtained from all participants.

Results

Participant characteristics A total of 391,199 participants were included in the main analysis (mean age, 56.9 years; 54.1% women). For type 2 diabetes, there were 26,690 cases. For cardiovascular disease, there were 38,098 cases of coronary artery disease and 11,358 cases of stroke. For cancer, there were 80,941 cases of overall cancer, 9251 cases of breast cancer, 5861 cases of colorectal cancer and 8970 cases of prostate cancer. In Table 1, HbA_{1c}, glucose, insulin therapy users and metformin therapy users were significantly lower in the high AMPK group than in the low group. No other significant differences in baseline characteristics between the two groups were found.

Association of AMPK score with glycaemic traits and type 2 diabetes Compared with participants with a low AMPK score (below median favouring higher HbA_{1c}), participants with a high AMPK score (above median) had lower HbA_{1c} (β -0.032%, 95% CI -0.035, -0.028; $p=2.34\times10^{-64}$), and lower random blood glucose (β -0.013 mmol/l, 95% CI -0.022, -0.005; $p=1.09\times10^{-3}$). High AMPK score (above median) was also associated with a decreased

risk of type 2 diabetes (OR 0.96; 95% CI 0.94, 0.99; $p=4.16\times10^{-3}$), as shown in Fig. 2a. AMPK quartiles were associated with a stepwise decrease in HbA_{1c} (quartile 2, -0.011%; 95% CI -0.016, -0.006; $p=2.70\times10^{-5}$; quartile 3, -0.028%; 95% CI -0.033, -0.022; $p=2.02\times10^{-25}$; and quartile 4, -0.047%; 95% CI -0.052, -0.042; $p=4.67\times10^{-70}$), and a corresponding stepwise decrease in the risk of type 2 diabetes (p for trend=4.18×10^{-3}, Fig. 2a). Genetically predicted reduction in HbA_{1c} (%) instrumented by AMPK variants was associated with a 61% decrease in the risk of type 2 diabetes (OR 0.39 per % reduction; 95% CI 0.20, 0.78; $p=7.69\times10^{-3}$) (Fig. 3).

Association of AMPK score with cardiovascular diseases High AMPK score (above median) was associated with a 3% lower risk of coronary artery disease (OR 0.97; 95% CI 0.95, 0.99; $p=5.69\times10^{-3}$; Fig. 2b), but not stroke (ESM Fig. 2a). AMPK quartile was associated with a stepwise decrease in the risk of coronary artery disease (p for trend=4.37×10^{-3}; Fig. 2b). Genetically predicted reduction in HbA_{1c} (%) instrumented by AMPK variants was associated with a 53% decrease in the risk of coronary artery disease (OR 0.47 per % reduction; 95% CI 0.26, 0.84; $p=0.01$) (Fig. 3).

Association of AMPK score with cancer High AMPK score (above median) was associated with lower risk of overall cancer (OR 0.98; 95% CI 0.96, 1.00; $p=0.01$ and p for trend=4.04×10^{-3}; Fig. 2c), but not with prostate cancer, breast cancer or colorectal cancer (ESM Fig. 2b–d). AMPK quartile was associated with prostate cancer (quartile 2, OR 0.91; 95% CI 0.85, 0.96; $p=1.61\times10^{-3}$; quartile 3, OR 0.93; 95% CI 0.88, 0.99; $p=0.02$), although the dose–response was unclear (ESM Fig. 2b). Genetically predicted reduction in HbA_{1c} (%) instrumented by AMPK variants was associated with a 44% decrease in the risk of overall cancer (OR 0.56 per % reduction; 95% CI 0.36, 0.85; $p=7.23\times10^{-3}$) (Fig. 3).

Sensitivity analysis by using a more stringent variant selection criterion A 1% reduction in HbA_{1c} instrumented by rs2732480 was associated with a decreased risk of type 2 diabetes (OR 0.11; 95% CI 0.02, 0.50; $p=4.08\times10^{-3}$) and coronary artery disease (OR 0.22; 95% CI 0.06, 0.81; $p=0.02$). The direction with overall cancer was consistent with the main analysis but with wider CI (OR 0.45; 95% CI 0.17, 1.14; $p=0.09$) (ESM Table 4).

External validation In external replication analyses, genetically predicted lower HbA_{1c} instrumented by AMPK variants was associated with decreased risk of type 2 diabetes (OR 0.11 per % reduction; 95% CI 0.04, 0.35; $p=1.78\times10^{-4}$) and coronary artery disease (OR 0.48 per % reduction; 95 CI 0.33, 0.72; $p=2.89\times10^{-4}$), but not with stroke, breast cancer or prostate cancer (ESM Table 5 and ESM Fig. 3a–e). The *Q* statistic suggested possible heterogeneity for the associations with type 2 diabetes, coronary artery disease, stroke and breast cancer.

Association of HbA_{1c} with cardiovascular disease and cancer risk in the UK Biobank using Mendelian randomisation ESM Table 6 shows that genetically predicted higher HbA_{1c} was associated with higher risk of coronary artery disease (OR 1.41; 95% CI 1.03, 1.93; $p=0.03$), and possibly with lower risk of overall cancer (OR 0.84; 95% CI 0.70, 1.01; $p=0.07$), but not with stroke, or any cancer subtype.

Discussion

To the best of our knowledge, this is one of the first Mendelian randomisation studies to ascertain the effects of metformin, based on AMPK variants, on cardiovascular diseases and cancer. Using a design more robust to immortal time biases and confounding, our study is consistent with previous pharmaco-epidemiological studies suggesting that metformin use may reduce coronary artery disease and overall cancer risk. We added some genetic evidence

that the putative cancer-protective effect of metformin via AMPK pathways is unlikely by glycaemic control.

A protective effect of metformin on cardiovascular health was observed in small randomised controlled trials using surrogate outcomes [32], the UK Prospective Diabetes Study post-trial analysis [33] and a recent meta-analysis [8], which were consistent with our findings. Although a genetically predicted reduction in HbA_{1c} is protective against coronary artery disease [14], it is apparent that metformin's protective effect is not solely due to its improvement in glycaemic profile given that these benefits are not clearly observed for all other classes of glucose-lowering medications [8], such as sulfonylureas and insulin [2, 34]. Metformin increases GDF-15, a stress-responsive cytokine which suppresses appetite and promotes weight loss [35], hence providing a potential mechanistic pathway by which metformin reduces cardiovascular disease risk. However, changes in GDF-15 were not clearly associated with coronary artery disease risk based on our previous Mendelian randomisation study [11]. On the contrary, sulfonylureas and insulin may lead to cardiotoxicity via weight gain, hypoglycaemia [34] or alteration of hormone levels [36].

The relation of metformin use with cancer risk is more controversial given the concern over immortal time bias [6]. Our study, where the start of 'exposure' is at birth, effectively removes this bias. As such, our study adds by showing that immortal time bias alone may not have explained the inverse relation of metformin use with cancer risk. Given that previous studies generally have ruled out the causal role of glycaemic traits in cancer risk [10, 37], possible mechanisms underlying the anti-cancer property of metformin are likely via pre-cursors of glycaemic traits or of glycaemic-independent pathways [38]. People without growth hormone appear to be protected against both diabetes and cancer [39]. This may suggest a possible pathway via growth hormone or the closely related insulin like growth

factor-1 [40]. Glycaemic-independent pathways may include inhibition of tumour-mesothelial cell interaction by suppressing hypoxia-inducible factor 1α and TGF-β signalling [41], immune-mediated responses via metabolic reprogramming of tumour-specific T cells [42] and GDF-15 overexpressing fibroblasts promoting the growth of tumour xenografts [43]. The examination of these potential mechanisms can be explored in further studies. Big data approaches, such as metabolomics, may also be warranted to better understand the full spectrum of effects of metformin, and hence to help identify the main pathways in which metformin confers the additional benefits on cardiovascular disease and cancer [44].

Although our study is more robust to confounding and immortal time bias than previous observational studies, there are limitations. First, whilst our study suggested that AMPK activation by metformin may protect against coronary artery disease, and possibly cancer, the estimates from this study cannot be used directly to infer the health impact of metformin given the differences in exposure time, where randomised controlled trials often consider short-term pharmacological treatment in contrast to the effects of lifelong exposures estimated by Mendelian randomisation [12]. Moreover, our study using AMPK variants may only predict the effect of metformin which acts on the AMPK activation pathways, and metformin may also have AMPK-independent pathways that could be explored in additional studies to fully capture the overall effect of metformin on cardiovascular disease and cancer [45]. Second, we used a lower threshold than genome-wide statistical significance to select AMPK variants as a proxy of metformin use to maximise total prediction of AMPK function by the genetic score. We reduced the possibility of false positives by cross-checking the variants' associations with HbA_{1c} in two independent studies. We also repeated the analysis with stringent variant selection criteria which gave a consistent conclusion. However, this may compromise the generalisability of the genetic score in other studies [46]. Third, we cannot rule out selection bias resulting from the recruitment of generally healthier

participants and survivors in the UK Biobank, which may bias the estimate towards the null. We also cannot rule out selection bias from competing risk before recruitment for diseases which share risk factors with other diseases that typically occur at younger ages, which could have biased estimates for stroke and prostate cancer to the null. Forth, the *Q* statistic suggested possible heterogeneity in some analyses. These heterogeneities may imply that multiple gene regions encoding subunits of AMPK may have different mechanisms of influencing the outcomes, and should be explored in future studies [28]. Fifth, given the pleiotropic effects of metformin and its association with multiple non-glycaemic makers [5], it would be difficult to identify a suitable negative control outcome. Nevertheless, we also assessed the impact of HbA_{1c} on these outcomes and found that HbA_{1c} unlikely explained all of the observed associations related to AMPK. Lastly, we could not exclude the possibility that metformin may reduce subtypes of cancer as the number of cases was not large enough for adequate statistical power, although the directions of effect for some cancer subtypes are similar to overall cancer. Few AMPK genetic variants were available for prostate cancer and breast cancer in the consortia and we were unable to create an overall genetic score in the associated analyses to increase statistical power. Together with possible selection biases embedded in these GWAS [47], these factors might explain the discrepancies between the estimates from the UK Biobank and from external consortia. Further investigations in large consortia on specific cancers may help verify the potential anti-cancer property of metformin.

Conclusion This Mendelian randomisation study provides some genetic evidence that AMPK activation by metformin may reduce coronary artery disease risk, and possibly overall cancer risk. Whether metformin can be repurposed for coronary artery disease and cancer should be explored in large randomised controlled trials.

Acknowledgements This research has been conducted using the UK Biobank Resource (www.ukbiobank.ac.uk) under application number 51001. Summary data on HbA_{1c} have been contributed by MAGIC investigators and have been downloaded from http://www.magicinvestigators.org/. Summary data on type 2 diabetes have been contributed by DIAGRAM investigators and have been downloaded from http://diagram-consortium.org/. Summary data on coronary artery disease have been contributed by CARDIoGRAMplusC4D investigators and have been downloaded from http://www.cardiogramplusc4d.org/. Summary data on stroke have been contributed by the MEGASTROKE investigators and have been downloaded from http://www.megastroke.org/. The MEGASTROKE project received funding from sources specified at http://www.megastroke.org/acknowledgments.html. Summary data on breast cancer have been contributed by BCAC investigators and have been downloaded from http://bcac.ccge.medschl.cam.ac.uk/bcacdata/. Summary data on prostate cancer have been contributed by the PRACTICAL consortium, CRUK, BPC3, CAPS and PEGASUS and have been downloaded from http://practical.icr.ac.uk/blog/. All studies and funders related to BCAC and PRACTICAL are listed in the electronic supplementary material.

Data availability The data generated and analysed during the current study are available from the corresponding author on reasonable request.

Funding This research is funded by the Seed Fund for Basic Research, the University of Hong Kong (No. 201811159054 to SLAY). SL is supported by the Bau Tsu Zung Bau Kwan Yeu Hing Research and Clinical Fellowship (*200008682.920006.20006.400.01), the University of Hong Kong. ICKW received research funding outside the submitted work from Amgen, Bristol-Myers Squibb, Pfizer, Janssen, Bayer, GSK, Novartis, the Hong Kong Research Grant Council, the Hong Kong Health and Medical Research Fund, the National

Institute for Health Research in England, the European Commission, and the National Health and Medical Research Council in Australia, and also received speaker fees from Janssen and Medice in the previous 3 years. The study sponsor/funder was not involved in the design of the study; the collection, analysis and interpretation of data; writing the report; and did not impose any restrictions regarding the publication of the report.

Authors' relationships and activities The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Contribution statement SL and SLAY designed the study, wrote the research plan and interpreted the results. SL undertook analyses with feedback from SLAY, CMS and ICKW. SL and SLAY wrote the manuscript with critical comments from CMS and ICKW. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. All authors gave final approval of the version to be published. SL is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Table 1 Baseline characteristics of the participants in the UK Biobank

Baseline characteristic	**AMPK score < median (n=194,634)**	**AMPK score ≥ median (n=196,565)**	***p* value**
Age at recruitment (years)	56.9±8.0	56.9±8.0	0.85
Female, No. (%)	105,048 (54.0)	106,632 (54.2)	0.08
Current smoker, No. (%)	19,869 (10.2)	19,689 (10.0)	0.09
Current alcohol drinker, No. (%)	181,839 (93.4)	183,332 (93.3)	0.22
BP (mmHg)			
Systolic	138.3±18.7	138.3±18.6	0.73
Diastolic	82.3±10.1	82.3±10.1	0.34
BMI (kg/m^2)	27.4±4.8	27.4±4.8	0.61
Education level Degree, No. (%)	88,727 (45.6)	89,717 (45.6)	0.94
Townsend deprivation index	-1.55±2.94	-1.56±2.93	0.21
HbA_{1c} (mmol/mol)	36.14±6.49	35.8±6.33	<0.001
HbA_{1c} (%)	5.46±0.59	5.43±0.58	<0.001
Random glucose (mmol/l)	5.13±1.23	5.11±1.20	<0.001
Current treatment, No. (%)			
Antihypertensive therapy	43,400 (22.3)	43,564 (22.2)	0.31
Insulin therapy	2197 (1.1)	2077 (1.1)	0.03
Metformin therapy	5465 (2.8)	5224 (2.7)	0.004

Values are mean ± SD

To convert values for HbA_{1c} (mmol/mol, IFCC unit) to percentage (NGSP unit), we used the master equation NGSP=(0.09148×IFCC)+2.152

To convert glucose from mmol/l to mg/dl, multiply by 18

Fig. 1 Study design of this Mendelian randomisation study (a) and its comparison with a randomised controlled trial (b)

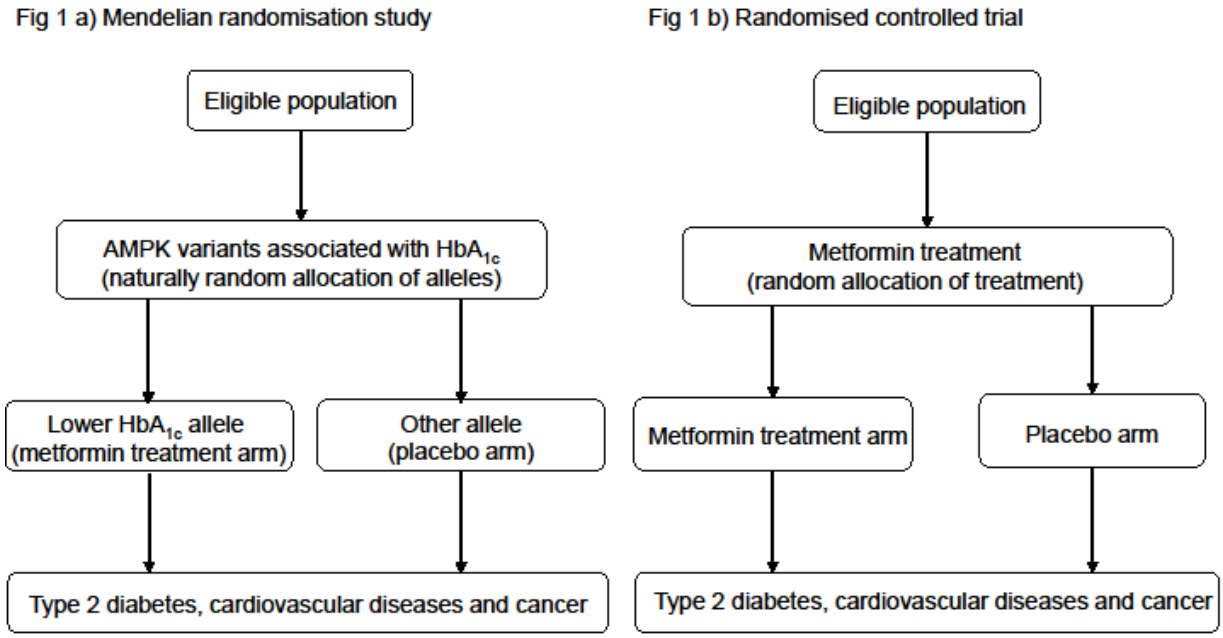
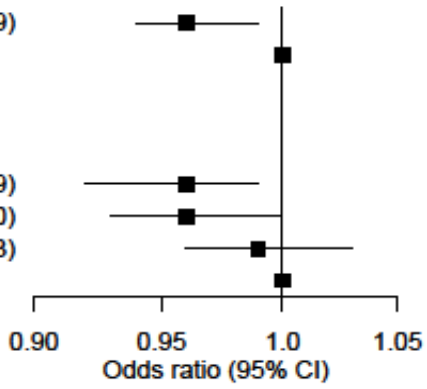


Fig. 1 Study design of this Mendelian randomisation study (a) and its comparison with a randomised controlled trial (b)

Fig. 2 Association of AMPK score with risk of type 2 diabetes (a), coronary heart disease (b) and overall cancer (c) in the UK Biobank. Boxes represent ORs and lines represent 95% CIs

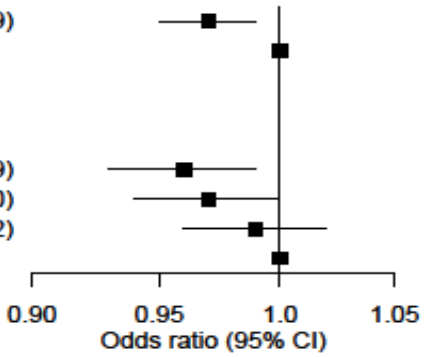
a) Type 2 diabetes

Group	No. of cases (%)	OR (95% CI)
Primary analysis		
AMPK score above median	13,167 (3.4)	0.96 (0.94, 0.99)
AMPK score below median	13,523 (3.5)	Reference
Dose−response analysis		
Quartile of AMPK score		
4	6613 (1.7)	0.96 (0.92, 0.99)
3	6554 (1.7)	0.96 (0.93, 1.00)
2	6711 (1.7)	0.99 (0.96, 1.03)
1	6812 (1.7)	Reference



b) Coronary artery disease

Group	No. of cases (%)	OR (95% CI)
Primary analysis		
AMPK score above median	18,882 (4.8)	0.97 (0.95, 0.99)
AMPK score below median	19,216 (4.9)	Reference
Dose−response analysis		
Quartile of AMPK score		
4	9530 (2.4)	0.96 (0.93, 0.99)
3	9352 (2.4)	0.97 (0.94, 1.00)
2	9521 (2.4)	0.99 (0.96, 1.02)
1	9695 (2.5)	Reference



c) Overall cancer

Group	No. of cases (%)	OR (95% CI)
Primary analysis		
AMPK score above median	40,383 (10.3)	0.98 (0.96, 1.00)
AMPK score below median	40,558 (10.4)	Reference
Dose−response analysis		
Quartile of AMPK score		
4	20,478 (5.2)	0.97 (0.95, 0.99)
3	19,905 (5.1)	0.96 (0.94, 0.98)
2	19,999 (5.1)	0.97 (0.95, 0.99)
1	20,559 (5.3)	Reference

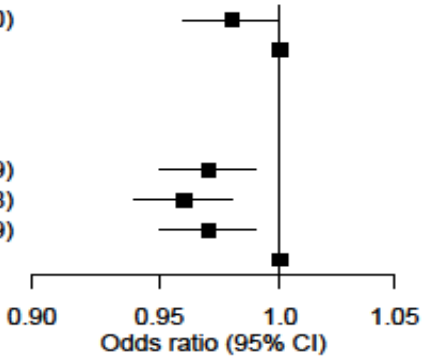
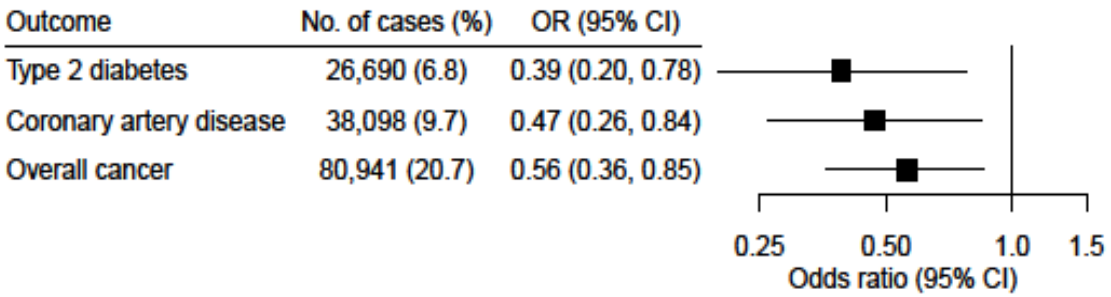


Fig. 3 The impact of genetically predicted reduction in HbA_{1c} (%) instrumented by AMPK variants on risk of type 2 diabetes, coronary artery disease and overall cancer in the UK Biobank. Boxes represent ORs and lines represent 95% CIs



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Electronic supplementary material

Evaluating the impact of AMPK activation, a target of metformin, on risk of cardiovascular diseases and cancer in the UK Biobank: a Mendelian randomisation study

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ESM Table 1 Variants included in AMP-activated protein kinase score and association with HbA_{1c} (%) in the Meta-Analyses of Glucose and Insulin-related traits Consortium, restricted to participants of European descent

Variant	Effect allele	Other allele	EAF	Effect size	Standard error	P MAGIC	P UK Biobank
rs11239944	A	G	0.825	-0.018	0.0061	3.05E-03	1.88E-04
rs2059409	T	C	0.045	-0.011	0.0047	2.17E-02	1.16E-02
rs1365964	G	A	0.801	-0.0057	0.0029	4.93E-02	6.91E-03
rs11884246	A	C	0.522	-0.0033	0.0017	4.64E-02	2.61E-02
rs6726126	A	G	0.518	-0.0038	0.0018	3.09E-02	3.38E-07
rs16858808	A	G	0.04	-0.025	0.012	3.22E-02	2.36E-03
rs17572109	A	G	0.208	-0.0055	0.002	5.18E-03	1.54E-05
rs7596500	G	T	0.982	-0.016	0.0074	3.16E-02	8.87E-03
rs3816560	C	T	0.221	-0.004	0.0019	3.19E-02	2.07E-05
rs10230736	G	A	0.862	-0.011	0.0038	4.44E-03	4.13E-04
rs1808593	T	G	0.827	-0.0049	0.0024	3.96E-02	3.83E-03
rs1563636	C	T	0.196	-0.0057	0.0025	2.18E-02	1.94E-03
rs7806203	C	T	0.345	-0.0039	0.002	4.74E-02	6.75E-03
rs7780461	T	C	0.118	-0.0063	0.0032	4.97E-02	1.16E-02
rs10259821	A	G	0.31	-0.0037	0.0019	4.71E-02	1.20E-03
rs1635527	C	G	0.525	-0.0038	0.0019	4.15E-02	4.35E-36
rs1859444	C	T	0.808	-0.0048	0.0024	4.39E-02	1.42E-22
rs1859443	G	A	0.856	-0.0058	0.0025	1.96E-02	6.27E-13
rs11168355	G	A	0.792	-0.0055	0.0022	1.14E-02	2.63E-43
rs11168359	A	G	0.119	-0.015	0.0032	1.61E-06	6.94E-91
rs12297820	A	G	0.119	-0.017	0.0029	1.26E-08	1.09E-43
rs10492081	A	G	0.81	-0.0071	0.0024	2.87E-03	9.20E-21
rs17614932	A	C	0.975	-0.012	0.0051	1.45E-02	2.70E-11
rs10875764	C	T	0.584	-0.0077	0.0038	4.45E-02	1.32E-13
rs7134565	C	T	0.469	-0.0035	0.0018	4.68E-02	3.79E-37
rs2732480*	A	C	0.425	-0.012	0.002	2.00E-09	1.07E-142
rs1489107	A	G	0.031	-0.014	0.0058	1.73E-02	2.16E-22
rs2932091	A	C	0.325	-0.0043	0.0021	3.79E-02	1.25E-03
rs10875801	C	T	0.825	-0.0089	0.0023	9.35E-05	5.95E-30
rs4760702	A	T	0.967	-0.011	0.0048	1.98E-02	1.16E-09
rs7959684	A	G	0.226	-0.005	0.0021	1.58E-02	1.30E-50
rs11168547	T	C	0.943	-0.0079	0.0026	2.28E-03	1.06E-26
rs12582586	T	C	0.212	-0.005	0.0024	3.58E-02	1.92E-04
rs10875814	G	A	0.733	-0.0051	0.0024	3.22E-02	1.34E-09
rs12582811	G	T	0.128	-0.0089	0.0033	7.23E-03	1.96E-06
rs11168643	A	G	0.034	-0.018	0.0061	2.89E-03	3.84E-08
rs10875843	A	G	0.033	-0.0093	0.0045	3.67E-02	4.79E-08
rs7975821	A	G	0.724	-0.0041	0.0021	4.77E-02	2.81E-07
rs17834622	A	G	0.346	-0.0064	0.0019	5.95E-04	1.10E-17
rs12830014	G	A	0.914	-0.0074	0.0037	4.71E-02	8.62E-04
rs10783277	C	T	0.674	-0.0045	0.0019	1.58E-02	4.35E-05
rs12322783	A	G	0.189	-0.0045	0.0023	4.82E-02	1.80E-08
rs17197593	T	C	0.053	-0.014	0.0062	2.23E-02	5.50E-05
rs1050187	C	T	0.704	-0.0042	0.0019	2.42E-02	1.71E-03

HbA_{1c} measured in percentage. For each variant, the effect allele is the allele associated with lower HbA_{1c}. Association are taken from Wheeler E *et al. PLoS Med.* EAF, effect allele frequency.
*Variant used in the sensitivity analysis, chosen based on a stringent variant selection criterion.

ESM Table 2 UK biobank's algorithmically defined disease outcomes

Data fields	Type 2 diabetes	Coronary artery disease	Stroke	Overall cancer	Breast cancer	Colorectal cancer	Prostate cancer
ICD-9 40013, 41203, 41205	250	410-414	430, 431, 434, 436	140-239	174-175	153.0-154.1	185
ICD-10 40001, 40002, 40006, 41202, 41204	E11	I20-I25	I60, I61, I63, I64	C00-C97	C50	C18-C20	C61
20001				data coding3	1002	1020	1044
20002	1223	1074, 1075	1081, 1086, 1491, 1583				
2453				1			
6150		1, 2	3				
20003	1140884600, 1140874686, 1141153254, 1141171646, 1141177600, 1141189090, 1140857584, 1140874706, 1140874664, 1140874674, 1140857494, 1140874744, 1140874646, 1141157284, 1140874658, 1141152590, 1141168660, 1141173882						
30750	≥ 6.5%, or 48 mmol/mol						
30740	≥ 11.1 mol/L						

ESM Table 3 The variants associated with HbA_{1c} (%) in the Meta-Analyses of Glucose and Insulin-related traits Consortium, restricted to participants of European descent

Variant	Effect allele	Other allele	EAF	Beta	Standard error	P value	Sample size
rs267738	T	G	0.7701	0.011	0.0019	2.59E-09	118146
rs857691	T	C	0.2715	0.019	0.0019	3.97E-25	121554
rs17509001	C	T	0.1576	0.018	0.0023	1.94E-15	121575
rs12621844	T	C	0.5999	0.01	0.0018	1.87E-08	88288
rs560887	C	T	0.6843	0.028	0.0018	1.48E-58	109489
rs7616006	A	G	0.5744	0.01	0.0017	5.07E-10	121507
rs9818758	A	G	0.2028	0.012	0.002	7.74E-10	121581
rs11708067	A	G	0.7542	0.013	0.0019	1.42E-12	119780
rs8192675	T	C	0.6906	0.011	0.0017	1.38E-11	119841
rs13134327	A	G	0.3335	0.013	0.0017	2.64E-15	119717
rs7756992	G	A	0.2862	0.012	0.0018	2.80E-12	118856
rs198846	G	A	0.8293	0.022	0.0022	1.18E-23	120479
rs11964178	A	G	0.5666	0.01	0.0016	6.38E-10	121505
rs592423	A	C	0.4566	0.009	0.0017	3.96E-08	107880
rs4607517	A	G	0.2017	0.031	0.0024	8.76E-38	86837
rs6474359	T	C	0.953	0.044	0.0053	1.50E-16	95687
rs4737009	A	G	0.2531	0.021	0.002	4.48E-27	120823
rs6980507	A	G	0.4014	0.01	0.0018	3.58E-08	108044
rs11558471	A	G	0.6745	0.015	0.0017	1.38E-19	121354
rs2383208	A	G	0.7992	0.014	0.0021	7.04E-12	113265
rs7040409	C	G	0.8953	0.028	0.0037	2.56E-14	106582
rs579459	C	T	0.2389	0.011	0.0019	9.42E-09	120555
rs4745982	T	G	0.8726	0.095	0.0056	2.87E-65	69523
rs17747324	C	T	0.2489	0.015	0.0023	6.12E-11	87696
rs3782123	C	A	0.3205	0.013	0.002	1.51E-10	105906
rs11603334	G	A	0.815	0.012	0.0021	6.85E-09	120520
rs10830963	G	C	0.2938	0.02	0.002	2.23E-23	100954
rs2110073	T	C	0.1097	0.015	0.0028	4.44E-08	119835
rs10774625	G	A	0.5056	0.009	0.0016	1.46E-08	121429
rs282587	G	A	0.1513	0.019	0.0027	1.70E-12	88316
rs9604573	A	G	0.2738	0.01	0.0018	9.60E-09	115096
rs11248914	T	C	0.647	0.014	0.0019	2.56E-14	85606
rs1558902	A	T	0.4128	0.01	0.0019	3.27E-08	88319
rs837763	T	C	0.5548	0.017	0.0016	1.68E-28	111180
rs9914988	A	G	0.7877	0.013	0.002	2.77E-11	121502
rs1046896	T	C	0.3162	0.028	0.0017	4.46E-64	123491
rs17533903	A	G	0.2428	0.015	0.0022	5.27E-12	119537
rs4820268	G	A	0.4606	0.016	0.0017	1.40E-22	109500

HbA_{1c} measured in percentage. For each variant, the effect allele is the allele associated with higher HbA_{1c}. Association are taken from Wheeler E *et al. PLoS Med.* EAF, effect allele frequency.

ESM Table 4 The impact of genetically predicted reduction in HbA_{1c} (%) instrumented by rs2732480 on type 2 diabetes, coronary artery disease and overall cancer in the UK Biobank

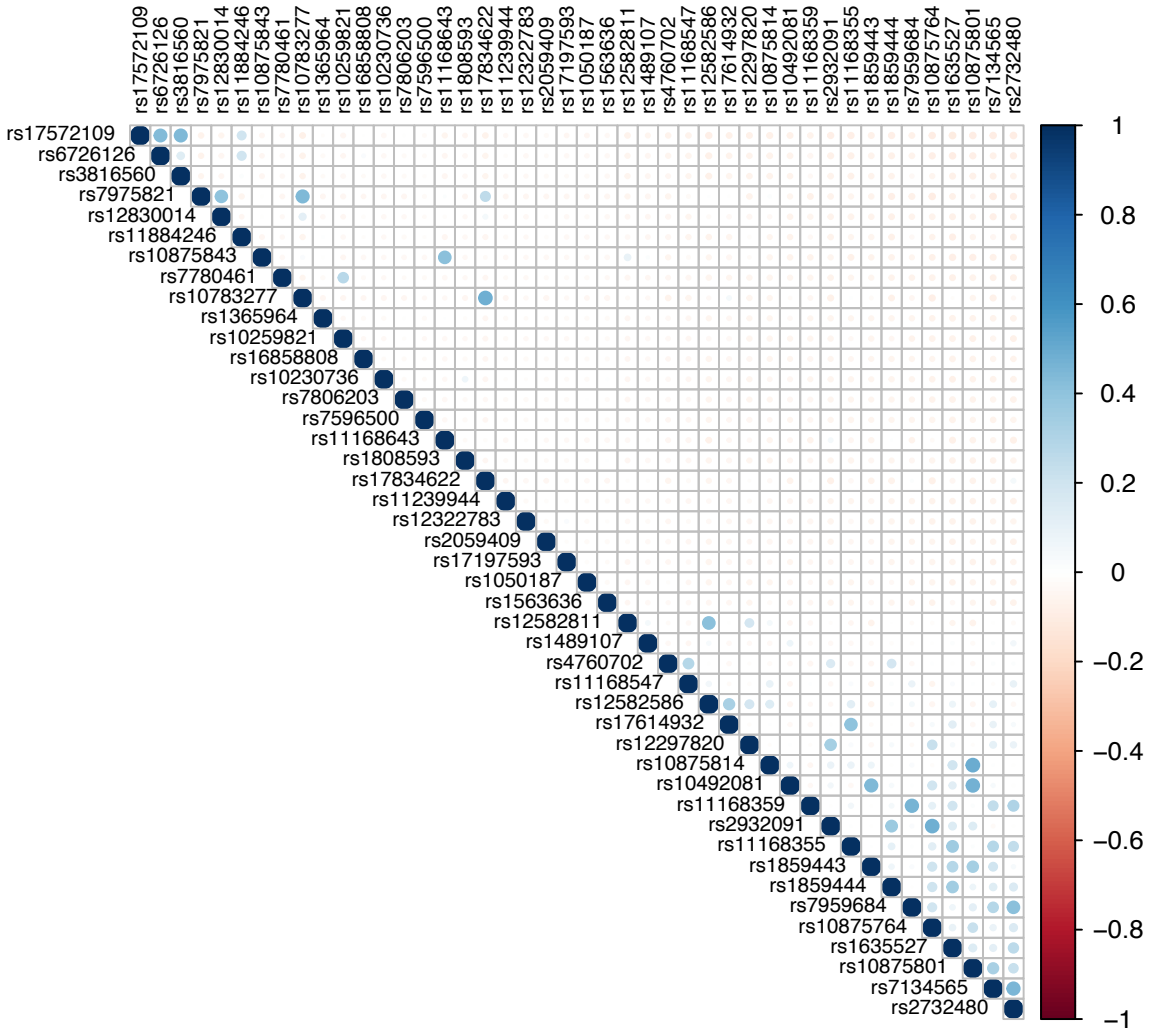
Outcome	**No. of case (%)**	**OR (95% CI)**	**P value**
Type 2 diabetes	26,690 (6.9)	0.11 (0.02 to 0.50)	4.08×10^{-3}
Coronary artery disease	38,098 (9.7)	0.22 (0.06 to 0.81)	0.02
Overall cancer	80,941(20.7)	0.45 (0.17 to 1.14)	0.09

ESM Table 5 The impact of genetically predicted reduction in HbA_{1c} (%) instrumented by AMPK variants on risk of type 2 diabetes, cardiovascular diseases and cancers

Outcome	Consortium	No. of case	No. of control	No. of variants	Inverse variance weighted		*Q* statistics	
					OR (95 % CI)	P value	Heterogeneity	P value
Type 2 diabetes	DIAGRAM	12,171	56,862	39	0.11 (0.04 to 0.35)	1.78×10^{-4}	123	6.09×10^{-11}
Coronary artery disease	CARDIoGRAMplusC4D	60,801	123,504	42	0.48 (0.33 to 0.72)	2.89×10^{-4}	80	2.45×10^{-4}
Stroke	MEGASTROKE	40,585	406,111	43	0.95 (0.56 to 1.61)	0.84	216	3.07×10^{-25}
Breast cancer	BCAC	122,977	105,974	15	0.61 (0.26 to 1.43)	0.25	41	2.12×10^{-4}
Prostate cancer	PRACTICAL	79,148	61,106	13	0.85 (0.41 to 1.78)	0.66	7	0.85

ESM Table 6 The impact of genetically predicted HbA_{1c} (%) on risk of cardiovascular diseases and cancers in the UK Biobank

Outcome	No. of case (%)	Method	OR (95% CI)	P value
Coronary artery disease	38,098 (9.7)	Inverse variance weighted	1.41 (1.03 to 1.93)	0.03
		Weighted median	1.17 (0.91 to 1.50)	0.23
		MR Egger	1.38 (0.76 to 2.50)	0.29
Stroke	11,358 (2.9)	Inverse variance weighted	1.24 (0.86 to 1.81)	0.25
		Weighted median	1.09 (0.72 to 1.65)	0.67
		MR Egger	0.99 (0.49 to 2.00)	0.98
Overall cancer	80,941(20.7)	Inverse variance weighted	0.84 (0.70 to 1.01)	0.07
		Weighted median	0.80 (0.67 to 0.96)	0.02
		MR Egger	0.77 (0.54 to 1.10)	0.16
Prostate cancer	8970 (2.2)	Inverse variance weighted	0.81 (0.52 to 1.28)	0.37
		Weighted median	0.96 (0.58 to 1.58)	0.87
		MR Egger	1.06 (0.45 to 2.46)	0.90
Breast cancer	9251 (2.4)	Inverse variance weighted	1.01 (0.61 to 1.67)	0.97
		Weighted median	1.41 (0.87 to 2.28)	0.16
		MR Egger	1.46 (0.57 to 3.73)	0.43
Colorectal cancer	5861 (1.5)	Inverse variance weighted	1.09 (0.67 to 1.79)	0.73
		Weighted median	0.82 (0.47 to 1.45)	0.50
		MR Egger	0.96 (0.38 to 2.44)	0.93

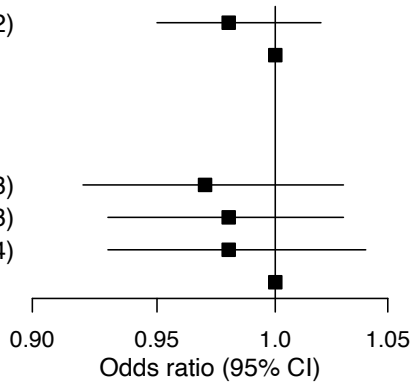


ESM Fig. 1 Linkage disequilibrium matrix for variants included in the AMP-activated protein kinase score

Values represent r^2 values, a measure of linkage disequilibrium. R^2 values range from 0 to1; with 0 representing complete equilibrium and 1 representing complete disequilibrium. Variants were included in the score if they had an r^2 value < 0.3 with other variants included in the score.

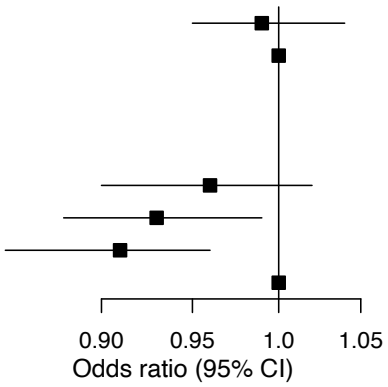
a) Stroke

Group	No. of cases (%)	OR (95% CI)
Primary analysis		
AMPK score above median	5659 (1.4)	0.98 (0.95, 1.02)
AMPK score below median	5699 (1.5)	Reference
Dose–response analysis		
Quartile of AMPK score		
4	2858 (0.7)	0.97 (0.92, 1.03)
3	2801 (0.7)	0.98 (0.93, 1.03)
2	2818 (0.7)	0.98 (0.93, 1.04)
1	2881 (0.7)	Reference



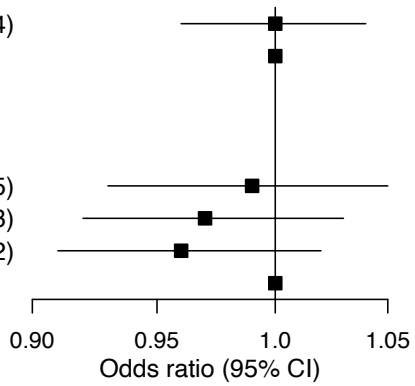
b) Prostate cancer

Group	No. of cases (%)	OR (95% CI)
Primary analysis		
AMPK score above median	4476 (1.1)	0.99 (0.95, 1.04)
AMPK score below median	4494 (1.1)	Reference
Dose–response analysis		
Quartile of AMPK score		
4	2289 (0.6)	0.96 (0.90, 1.02)
3	2187 (0.6)	0.93 (0.88, 0.99)
2	2138 (0.5)	0.91 (0.85, 0.96)
1	2356 (0.6)	Reference

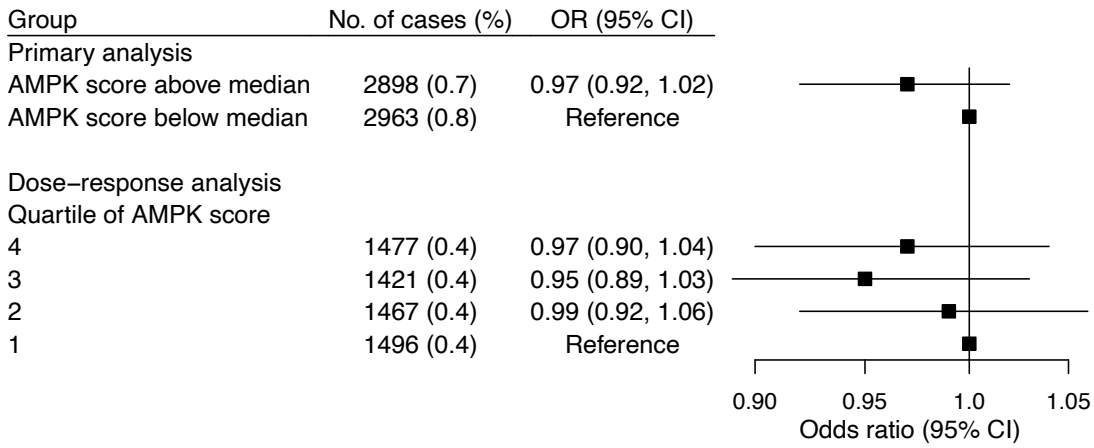


c) Breast cancer

Group	No. of cases (%)	OR (95% CI)
Primary analysis		
AMPK score above median	4656 (1.2)	1.00 (0.96, 1.04)
AMPK score below median	4595 (1.2)	Reference
Dose–response analysis		
Quartile of AMPK score		
4	2373 (0.6)	0.99 (0.93, 1.05)
3	2283 (0.6)	0.97 (0.92, 1.03)
2	2253 (0.6)	0.96 (0.91, 1.02)
1	2342 (0.6)	Reference

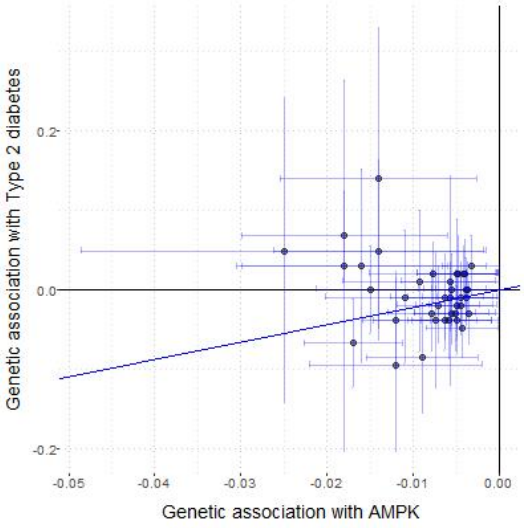


d) Colorectal cancer

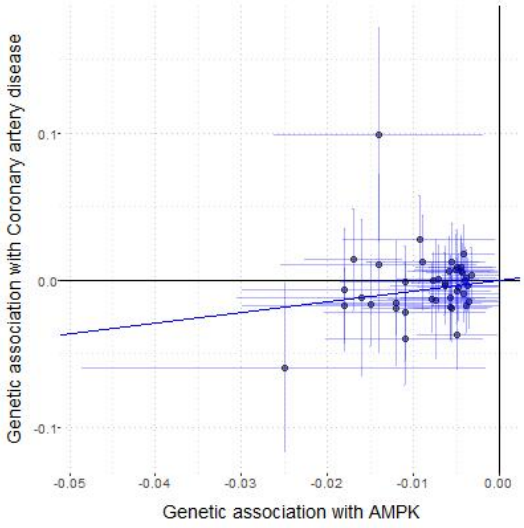


ESM Fig. 2 Association of AMP-activated protein kinase score with risk of stroke (a), prostate cancer (b), breast cancer (c), colorectal cancer (d) in the UK Biobank. Boxes represent ORs and lines represent 95% CIs

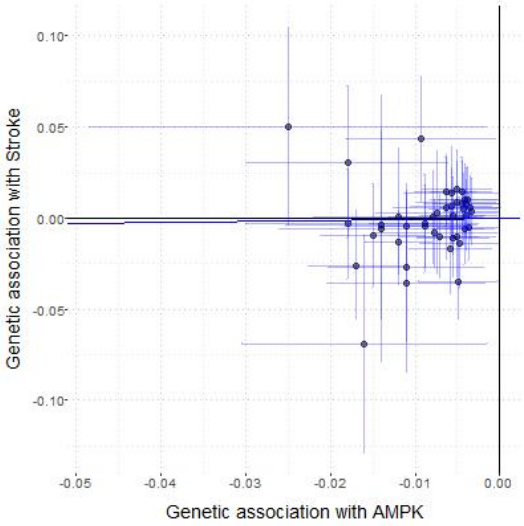
a) Type 2 diabetes



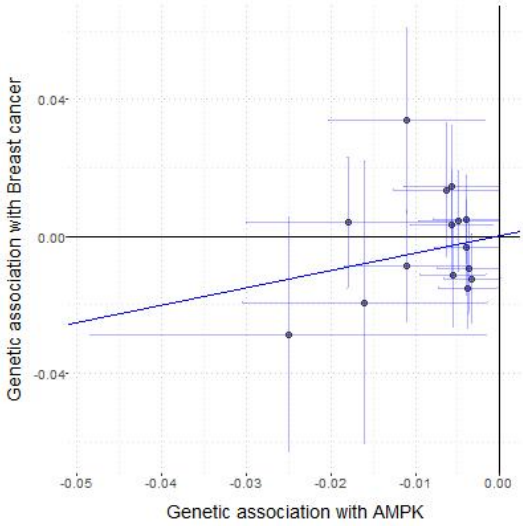
b) Coronary artery disease

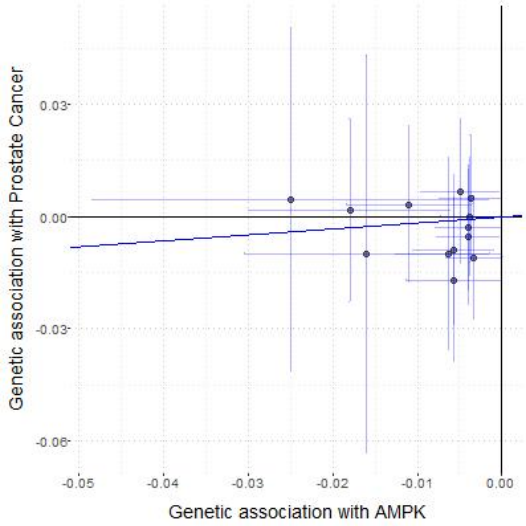


c) Stroke



d) Breast caner





e) Prostate cancer

ESM Fig. 3 Genetic associations instrumented by genetic variants with HbA_{1c} against type 2 diabetes (a), coronary artery disease (b), stroke (c), breast cancer (d), prostate cancer (e)

Genetic associations with HbA_{1c} (per allele decrease in HbA_{1c}) were estimated in participant of European ancestry only and obtained from the Meta-Analyses of Glucose and Insulin-related traits consortium, Wheeler et al (2017);[1] Genetic associations with type 2 diabetes risk (per allele log odds ratio) were obtained from the Diabetes Genetics Replication And Meta-analysis consortium, Morris et al (2012).[2] Genetic associations with coronary artery disease risk (per allele log odds ratio) were obtained the CARDIoGRAMplusC4D 1000 Genomes based genome wide association study, Nikpay et al (2015).[3] Genetic associations with stroke risk (per allele log odds ratio) were obtained from the MEGASTROKE consortium, Malik et al (2018);[4] Genetic associations with breast cancer risk (per allele log odds ratio) were obtained from the Breast Cancer Association Consortium, Michailidou et al (2017).[5] Genetic associations with prostate cancer risk (per allele log odds ratio) were obtained from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome consortium, Schumacher et al (2018).[6]

Acknowledgements related to BCAC and PRACTICAL

The breast cancer genome-wide association analyses are supported by the Government of Canada through Genome Canada and the Canadian Institutes of Health Research (CIHR), the 'Ministère de l'Économie, de la Science et de l'Innovation du Québec' through Genome Québec and grant PSR-SIIRI-701, the National Institutes of Health (NIH) (U19 CA148065, X01HG007492), Cancer Research UK (C1287/A10118, C1287/A16563, C1287/A10710) and The European Union (HEALTH-F2-2009-223175 and H2020 633784 and 634935).

The prostate cancer genome-wide association analyses are supported by the CIHRanadian Institutes of Health Research; the European Commission's Seventh Framework Programme grant agreement No.° 223175 (HEALTH-F2-2009-223175); Cancer Research UK Grants C5047/A7357, C1287/A10118, C1287/A16563, C5047/A3354, C5047/A10692 and, C16913/A6135; and the National Institute of Health (NIH) Cancer Post-Cancer GWAS initiative grant: No. 1 U19 CA 148537-01 (the GAME-ON initiative). We would also like to thank the following for funding support: the Institute of Cancer Research and the Everyman Campaign, the Prostate Cancer Research Foundation, the Prostate Research Campaign UK (now PCUK), the Orchid Cancer Appeal, Rosetrees Trust, the National Cancer Research Network UK, and the National Cancer Research Institute (NCRI) UK. We are grateful for the support of NIHR funding to the NIHR Biomedical Research Centre at the Institute of Cancer Research and the Royal Marsden NHS Foundation Trust. The Prostate Cancer Program of the Cancer Council Victoria also acknowledges grant support from the National Health and Medical Research Council, Australia (126402, 209057, 251533, , 396414, 450104, 504700, 504702, 504715, 623204, 940394, 614296,), VicHealth, the Cancer Council Victoria, the Prostate Cancer Foundation of Australia, the Whitten Foundation, PricewaterhouseCoopers and Tattersall's. EAO, DMK and EMK acknowledge the Intramural Program of the National Human Genome Research Institute for their support. Genotyping of the OncoArray was

funded by the US National Institutes of Health (NIH) (U19 CA 148537 for the ELucidating Loci Involved in Prostate cancer SuscEptibility [ELLIPSE] project and X01HG007492 to the Center for Inherited Disease Research (CIDR) under contract number HHSN268201200008I) and by Cancer Research UK grant A8197/A16565. Additional analytic support was provided by NIH NCI U01 CA188392 (principal investigator: Schumacher). Funding for the iCOGS infrastructure came from: the European Community's Seventh Framework Programme under grant agreement No. 223175 (HEALTH-F2-2009-223175) (COGS), Cancer Research UK (C1287/A10118, C1287/A 10710, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565), the NIHational Institutes of Health (CA128978), the and Post-Cancer GWAS initiative (1U19 CA148537, 1U19 CA148065 and 1U19 CA148112 —the GAME-ON initiative), the Department of Defence (W81XWH-10-1- 0341), the Canadian Institutes of Health Research (CIHR) for the CIHR Team in Familial Risks of Breast Cancer, the Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund. The BPC3 was supported by the US National Institutes of HealthNIH, National Cancer Institute (cooperative agreements U01- CA98233 to DJH, U01-CA98710 to SMG, U01-CA98216 to E.R., and U01-CA98758 to BEH, and the Intramural Research Program of the NIH/National Cancer Institute, Division of Cancer Epidemiology and Genetics). The CAPS GWAS study was supported by the Swedish Cancer Foundation (grant Nos 09-0677, 11-484, 12-823), the Cancer Risk Prediction Center (CRisP; www.crispcenter.org) and, a Linneus Centre (Contract ID 70867902) financed by the Swedish Research Council, Swedish Research Council (grant No. K2010-70X-20430-04-3, 2014-2269). PEGASUS was supported by the Intramural Research Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of HealthNIH.

Supplementary References

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