Genetically-proxied therapeutic inhibition of antihypertensive drug targets and risk of common cancers

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Abstract

Background: Epidemiological studies have reported conflicting findings on the potential adverse effects of long-term antihypertensive medication use on cancer risk. Naturally occurring variation in genes encoding antihypertensive drug targets can be used as proxies for these targets to examine the effect of their long-term therapeutic inhibition on disease outcomes.

Methods: Single-nucleotide polymorphisms (SNPs) in ACE, ADRB1, and SLC12A3 associated (P < 5.0 x 10^-8) with systolic blood pressure in genome-wide association studies (GWAS) were used to proxy inhibition of angiotensin-converting enzyme (ACE), β-1 adrenergic receptor (ADRB1), and sodium-chloride symporter (NCC), respectively. Summary genetic association estimates for these SNPs were obtained from GWAS consortia for the following cancers: breast (122,977 cases, 105,974 controls), colorectal (58,221 cases, 67,694 controls), lung (29,266 cases, 56,450 controls), and prostate (79,148 cases, 61,106 controls). Replication analyses were performed in the FinnGen consortium (1,573 colorectal cancer cases, 120,006 controls). Inverse-variance weighted random-effects models were used to examine associations between genetically-proxied inhibition of these drug targets and risk of cancer. Multivariable Mendelian randomization and colocalisation analyses were employed to examine robustness of findings to violations of Mendelian randomization assumptions.

Results: Genetically-proxied ACE inhibition equivalent to a 1 mmHg reduction in systolic blood pressure was associated with increased odds of colorectal cancer (OR 1.13, 95% CI 1.06-1.22; P = 3.6 x 10^-4). This finding was replicated in the FinnGen consortium (OR 1.40, 95% CI 1.02-1.92; P = 0.035). There was little evidence of association of genetically-proxied ACE inhibition with risk of breast cancer (OR 0.98, 95% CI 0.94-1.02, P = 0.35), lung cancer (OR 1.01, 95% CI 0.92-1.10; P = 0.93), or prostate cancer (OR 1.06, 95% CI 0.99-1.13; P = 0.08). Genetically-proxied inhibition of ADRB1 and NCC were not associated with risk of these cancers.

Conclusion: Genetically-proxied long-term ACE inhibition was associated with an increased risk of colorectal cancer, warranting comprehensive evaluation of the safety profiles of ACE inhibitors in...
clinical trials with adequate follow-up. There was little evidence to support associations across other
drug target-cancer risk analyses, consistent with findings from short-term randomised controlled trials
for these medications.
Background

Angiotensin-converting enzyme (ACE) inhibitors are commonly prescribed antihypertensive medications[1]. These medications lower blood pressure by inhibiting the conversion of angiotensin I to angiotensin II, a vasoconstrictor and the primary effector molecule of the renin-angiotensin system (RAS). Though clinical trials have supported the relative safety of these medications in the short-term (median follow-up of 3.5 years), concerns have been raised that long-term use of these medications could increase risk of cancer [2, 3]. These safety concerns relate to the multifaceted role of ACE which cleaves various other substrates beyond angiotensin I, including several peptides that have proliferative effects. For example, ACE inhibition leads to the accumulation of bradykinin, an inflammatory mediator involved in tumour growth and metastasis[4]. In addition, substance P is elevated in ACE inhibitor users which can promote tumour proliferation, migration, and angiogenesis [5, 6].

Some observational epidemiological studies have suggested potential adverse effects of long-term use of these drugs on risk of common cancers (i.e. breast, colorectal, lung, and prostate) [7-10], though findings have been largely inconsistent [11-13]. Interpretation of the epidemiological literature is challenging for several reasons. First, pharmaco-epidemiological studies are susceptible to residual confounding due to unmeasured or imprecisely measured confounders, including those related to indication[14]. Second, several studies examining ACE inhibitor use and cancer risk have included prevalent drug users which can introduce bias because prevalent users are “survivors” of the early period of pharmacotherapy and because covariates at study entry can be influenced by prior medication use[12, 15-18]. Third, some prior studies may have suffered from time-related biases, including immortal time bias which can arise because of misalignment of the start of follow-up, eligibility, and treatment assignment of participants[15, 16, 18, 19]. These biases can produce illusory results in favour of the treatment group while other biases often pervasive in the pharmaco-epidemiological literature (e.g. detection bias due to more intensive clinical monitoring and testing of individuals receiving treatment) can alternatively generate upward-biased effect estimates among those receiving treatment.
Along with ACE inhibitors, β blockers and thiazide diuretics are commonly prescribed antihypertensive medications that lower blood pressure through pathways independent to that of ACE (i.e. β blockers bind to β-adrenergic receptors, inhibiting the binding of norepinephrine and epinephrine to these receptors; thiazide diuretics promote sodium and water excretion by inhibiting sodium reabsorption in renal tubules)[4]. In contrast to ACE inhibitors, some in vitro and epidemiological studies have suggested potential chemo-preventive effects of these medications on cancer risk though findings have been inconclusive [20-28].

Naturally occurring variation in genes encoding antihypertensive drug targets can be used as proxies for these targets to examine the effect of their therapeutic inhibition on disease outcomes (“Mendelian randomization”) [29, 30]. Such an approach should be less prone to conventional issues of confounding as germline genetic variants are randomly assorted at meiosis. In addition, Mendelian randomization analysis permits the effect of long-term modulation of drug targets on cancer risk to be examined. Drug-target Mendelian randomization can therefore be used to mimic the effect of pharmacologically modulating a drug target in clinical trials and has been used previously to anticipate clinical benefits and adverse effects of therapeutic interventions[31-34].

We used a Mendelian randomization approach to examine the effect of long-term inhibition of the drug targets for ACE inhibitors (ACE; angiotensin-converting enzyme), β blockers (ADRB1; beta-1 adrenergic receptor), and thiazide diuretic agents (NCC; sodium-chloride symporter) on risk of overall and subtype-specific breast, colorectal, lung, and prostate cancer.

Methods

Study populations

For primary analyses, summary genetic association data were obtained from four cancer genome-wide association study (GWAS) consortia. Summary genetic association estimates for overall and
oestrogen receptor-stratified breast cancer risk in up to 122,977 cases and 105,974 controls were obtained from the Breast Cancer Association Consortium (BCAC)[35]. Summary genetic association estimates for overall and site-specific colorectal cancer risk in up to 58,221 cases and 67,694 controls were obtained from an analysis of the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), ColoRectal Transdisciplinary Study (CORECT), and Colon Cancer Family Registry (CCFR)[36]. Summary genetic association estimates for overall and histological subtype-stratified lung cancer risk in up to 29,266 cases and 56,450 controls were obtained from an analysis of the Integrative Analysis of Lung Cancer Risk and Etiology (INTEGRAL) team of the International Lung Cancer Consortium (ILCCO) [37]. Summary genetic association estimates for overall and advanced prostate cancer risk in up to 79,148 cases and 61,106 controls were obtained from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium[38]. These analyses were restricted to participants of European ancestry.

For replication analyses, summary genetic association data were obtained on 1,573 colorectal cancer cases and 120,006 controls of European ancestry from the Finngen consortium. We also examined whether findings could be extended to individuals of East Asian ancestry by obtaining summary genetic association data on 23,572 colorectal cancer cases and 48,700 controls of East Asian ancestry from a GWAS meta-analysis of the Asia Colorectal Cancer Consortium and the Korean National Cancer Center CRC Study 2[39].

Further information on statistical analysis, imputation, and quality control measures for these studies is available in the original publications. All studies contributing data to these analyses had the relevant institutional review board approval from each country, in accordance with the Declaration of Helsinki, and all participants provided informed consent.

Instrument construction

To generate instruments to proxy ACE, ADRB1, and NCC inhibition, we pooled summary genetic association data from two previously published GWAS of systolic blood pressure (SBP) using
inverse-variance weighted fixed-effects models in METAL[40]. The first GWAS was a meta-analysis of ≤ 757,601 individuals of European descent in the UK Biobank and International Consortium of Blood Pressure-Genome Wide Association Studies (ICBP)[41]. The second GWAS was performed in 99,785 individuals in the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, of whom the majority (81.0%) were of European ancestry[42]. Both GWAS were adjusted for age, sex, body mass index (BMI), and antihypertensive medication use. Estimates that were genome-wide significant ($P < 5.0 x 10^{-8}$) in pooled analyses (N ≤ 857,386) and that showed concordant direction of effect across both GWAS were then used to generate instruments.

To proxy ADRB1 inhibition, 8 SNPs associated with systolic blood pressure at genome-wide significance and within ± 100 kb windows from $ADRB1$ were obtained. To proxy NCC inhibition, 1 SNP associated with systolic blood pressure at genome-wide significance and within a ± 100 kb window from $SLC12A3$ (alias for $NCC$) was obtained. For both of these drug targets, SNPs used as proxies were permitted to be in weak linkage disequilibrium ($r^2 < 0.10$) with each other to increase the proportion of variance in each respective drug target explained by the instrument, maximising instrument strength.

Since pooled GWAS estimates were obtained from analyses adjusted for BMI, which could induce collider bias, we also examined constructing instruments using summary genetic association data from a previous GWAS of systolic blood pressure in 340,159 individuals in UK Biobank without adjustment for BMI or antihypertensive medication use (Supplementary Table 1) [43].

We explored construction of genetic instruments to proxy ACE inhibition using two approaches: i) by obtaining genome-wide significant variants in weak linkage disequilibrium ($r^2 < 0.10$) in or within ± 100 kb from $ACE$ that were associated with systolic blood pressure in previously described pooled GWAS analyses (resulting in two SNPs) and ii) by obtaining genome-wide significant variants in weak linkage disequilibrium ($r^2 < 0.10$) in or within ± 100 kb from $ACE$ that were associated with serum ACE concentrations in a GWAS of 4,174 participants in the Outcome Reduction with Initial Glargine INtervention (ORIGIN) study (resulting in 14 SNPs) [44]. 46.6% of participants in the ORIGIN study were of European ancestry and 53.4% were of Latin American ancestry. Effect allele
frequencies for these 14 SNPs were broadly similar across both ancestries (Supplementary Table 2).

We then compared the proportion of variance in either systolic blood pressure or serum ACE concentrations explained ($r^2$) across each respective instrument to prioritise the primary instrument to proxy ACE inhibition. The serum ACE concentrations instrument ($r^2 = 0.34-0.39$, $F= 2,156.5-2,594.9$) was prioritised because of stronger instrument strength as compared to the systolic blood pressure instrument ($r^2 =0.02$, $F=128.5$).

To validate the serum ACE concentrations instrument, we examined the association between genetically-proxied ACE inhibition and i) systolic blood pressure, ii) risk of stroke in the MEGASTROKE consortium (40,585 cases; 406,111 controls of European ancestry), iii) risk of coronary artery disease in the CARDIoGRAMplusC4D consortium (60,801 cases; 123,504 controls, 77% of whom were of European ancestry), and iv) risk of type 2 diabetes in the DIAGRAM consortium (N=74,124 cases; 824,006 controls of European ancestry) and compared the direction of effect estimates obtained with those reported for ACE inhibitor use in meta-analyses of randomised controlled trials[45-47]. Likewise, we validated ADRB1 and NCC instruments by examining the association between inhibition of these targets and risk of stroke and coronary artery disease, as reported in meta-analyses of clinical trials[47].

For analyses in individuals of East Asian ancestry, 1 cis-acting variant (rs4343) associated with ACE activity ($P = 3.0 \times 10^{-25}$) in a GWAS of 623 individuals with young onset hypertension of Han Chinese descent was obtained[48]. In the Japanese Biobank (N=136,597), the A allele of rs4343 has previously been shown to associate with lower SBP (-0.26 mmHg SBP, 95% CI -0.11 to -0.42; $P = 6.7 \times 10^{-4}$)[49]. This variant explained 0.008% of the variance of SBP ($F=11.6$).

**Mendelian randomization primary and sensitivity analyses**

Inverse-variance weighted random-effects models were employed to estimate causal effects of genetically-proxied drug target inhibition on cancer risk. These models were adjusted for weak linkage disequilibrium between SNPs ($r^2 < 0.10$) with reference to the 1000 Genomes Phase 3
reference panel[50, 51]. If under-dispersion in causal estimates generated from individual genetic
variants was present, the residual standard error was set to 1.

Mendelian randomization analysis assumes that the genetic instrument used to proxy a drug target (i)
is associated with the drug target (“relevance”), (ii) does not share a common cause with the outcome
(“exchangeability”), and (iii) affects the outcome only through the drug target (“exclusion
restriction”).

We tested the “relevance” assumption by generating estimates of the proportion of variance of each
drug target explained by the instrument ($r^2$) and F-statistics. F-statistics can be used to examine
whether results are likely to be influenced by weak instrument bias, i.e., reduced statistical power
when an instrument explains a limited proportion of the variance in a drug target. As a convention, an
F-statistic of at least 10 is indicative of minimal weak instrument bias[52].

We evaluated the “exclusion restriction” assumption by performing various sensitivity analyses. First,
we performed colocalisation to examine whether drug targets and cancer endpoints showing nominal
evidence of an association in MR analyses ($P < 0.05$) share the same causal variant at a given locus.
Such an analysis can permit exploration of whether drug targets and cancer outcomes are influenced
by distinct causal variants that are in linkage disequilibrium with each other, indicative of horizontal
pleiotropy (an instrument influencing an outcome through pathways independent to that of the
exposure), a violation of the exclusion restriction criterion [53]. Colocalisation analysis was
performed by generating ± 300 kb windows from the top SNP used to proxy each respective drug
target. As a convention, a posterior probability of $\geq 0.80$ was used to indicate support for a
configuration tested. An extended description of colocalisation analysis including assumptions of this
method is presented in Supplementary Material.

For analyses showing evidence of colocalisation across drug target and cancer endpoint signals, we
then examined whether there was evidence of an association of genetically-proxied inhibition of that
target with previously reported risk factors for the relevant cancer endpoint (e.g. body mass index,
low-density lipoprotein cholesterol, total cholesterol, iron, and insulin-like growth factor 1 for
colorectal cancer risk) [54-60]. If there was evidence for an association between a genetically-proxied drug target and previously reported risk factor ($P < 0.05$), which could suggest the presence of horizontal pleiotropy, multivariable Mendelian randomization can then be used to examine the association of drug target inhibition in relation to cancer risk, accounting for this risk factor[61].

Finally, iterative leave-one-out analysis was performed iteratively removing one SNP at a time from instruments to examine whether findings were driven by a single influential SNP.

To account for multiple testing across primary drug target analyses, a Bonferroni correction was used to establish a $P$-value threshold of $< 0.0014$ (false positive rate = 0.05/36 statistical tests [3 drug targets tested against 12 cancer endpoints]).

*Colon transcriptome-wide Mendelian randomization analysis*

To explore potential mechanisms governing associations and to further evaluate potential violations of Mendelian randomization assumptions, we examined associations of genetically-proxied ACE inhibition with gene expression profiles in normal (i.e. non-neoplastic) colon tissue samples. Gene expression analysis was performed using data from the University of Barcelona and the University of Virginia Genotyping and RNA Sequencing Project (BarcUVa-Seq)[62]. This analysis was restricted to 445 individuals (mean age 60 years, 64% female, 95% of European ancestry) who participated in a Spanish colorectal cancer risk screening programme who obtained a normal colonoscopy result (i.e. macroscopically normal colon tissue, with no malignant lesions). Further information on RNA-Seq data processing and quality control is presented in *Supplementary Material*.

To perform transcriptome-wide analyses, weighted genetic risk scores (wGRS) to proxy serum ACE concentrations were constructed using 14 ACE SNPs in Plink v1.9[63]. Expression levels for 21,482 genes (expressed as inverse normal transformed trimmed mean of M-values) were regressed on the standardised wGRS and adjusted for sex, the top two principal components of genetic ancestry, sequencing batch, probabilistic estimation of expression residuals (PEER) factors, and colon
anatomical location. To account for multiple testing, a Bonferroni correction was used to establish a P-value threshold of $< 2.33 \times 10^{-6}$ (false positive rate = 0.05/21,482 statistical tests).

Bioinformatic follow-up of findings from transcriptome-wide analysis was performed to further interrogate downstream perturbations of the ACE wGRS on gene expression profiles using gene-set enrichment analysis and co-expression network analysis. In brief, these methods can either evaluate whether expression levels of genes associated with the ACE wGRS are enriched in relation to an *a priori* defined set of genes based on curated functional annotation (gene-set enrichment analysis) or permit the identification of clusters of genes (termed “modules”) which show a coordinated expression pattern associated with the wGRS (co-expression network analysis). Further information on gene-set enrichment and co-expression network analysis is presented in Supplementary Material.

**Results**

Across the 3 drug targets that we examined, conservative estimates of F-statistics for their respective genetic instruments ranged from 269.1 to 2,156.5, suggesting that our analyses were unlikely to suffer from weak instrument bias. Characteristics of genetic variants in *ACE*, *ADRB1*, and *SLC12A3* used to proxy each pharmacological target are presented in Table 1. Estimates of $r^2$ and F-statistics for each target are presented in Supplementary Table 3.

**Instrument validation**

Findings from genetic instrument validation analyses for drug targets were broadly concordant (i.e. in direction of effect) with findings from meta-analyses of randomised trials for these medications. Genetically-proxyed ACE inhibition was associated with lower SBP (mmHg per SD lower serum ACE concentration: $-0.40$, 95% CI $-0.21$ to $-0.59$, $P = 4.2 \times 10^{-5}$) and a lower risk of type 2 diabetes (OR equivalent to 1 mmHg lower SBP: $0.90$, 95% CI $0.85$-$0.95$, $P = 1.3 \times 10^{-4}$). There was weak evidence
for an association of genetically-proxied ACE inhibition with lower risk of stroke (OR 0.94, 95% CI 0.88-1.01; \( P = 0.06 \)) and coronary artery disease (OR 0.95, 95% CI 0.89-1.02; \( P = 0.16 \)).

Genetically-proxied ADRB1 inhibition was associated with lower risk of coronary artery disease (per 1 mmHg lower SBP: OR 0.95, 95% CI 0.92-0.98; \( P = 1.5 \times 10^{-3} \)) but there was little evidence of association with risk of stroke (OR 1.03, 95% CI 0.99-1.07; \( P = 0.18 \)).

Genetically-proxied NCC inhibition was associated with lower risk of coronary artery disease (per 1 mmHg lower SBP: OR 0.81, 95% CI 0.81, 95% CI 0.71-0.93, \( P = 3.2 \times 10^{-3} \)) and was weakly associated with lower risk of stroke (OR 0.89, 95% CI 0.78-1.02; \( P = 0.10 \)).

Genetically-proxied ACE inhibition and cancer risk

Genetically-proxied ACE inhibition was associated with an increased odds of colorectal cancer (OR equivalent to 1 mmHg lower SBP: 1.13, 95% CI 1.06-1.22; \( P = 3.6 \times 10^{-4} \)). Likewise, in analyses using SBP SNPs in ACE, genetically-proxied SBP lowering via ACE inhibition was associated with an increased odds of colorectal cancer (OR equivalent to 1 mmHg lower SBP: 1.11, 95% CI 1.04-1.18; \( P = 1.3 \times 10^{-3} \)). When scaled to represent SBP lowering achieved in clinical trials of ACE inhibitors for primary hypertension (equivalent to 8 mmHg lower SBP), this represents an OR of 2.74 (95% CI 1.58-4.76)[64]. In site-specific analyses, this association was stronger for colon cancer risk (OR 1.18, 95% CI 1.07-1.31; \( P = 9.7 \times 10^{-4} \)) than rectal cancer risk (OR 1.07, 95% CI 0.97-1.18; \( P = 0.16 \)). Similar associations were found across risk of proximal colon cancer (OR 1.23, 95% CI 1.10-1.37; \( P = 1.9 \times 10^{-4} \)) and distal colon cancer (OR 1.15, 95% CI 1.03-1.27; \( P = 0.01 \)).

Colocalisation analysis suggested that serum ACE and colorectal cancer associations had a 91.4% posterior probability of sharing a causal variant within the ACE locus (Supplementary Table 4). Regional Manhattan plots examining the association of all SNPs ± 300 kb from the top SNP for serum ACE concentrations (rs4343) for their association with serum ACE concentrations (Figure 1) and with colorectal cancer risk (Figure 2) did not appear to support the presence of two or more independent causal variants driving associations across either trait.
In Mendelian randomization analyses examining the association of genetically-proxied ACE inhibition with previously reported colorectal cancer risk factors, there was little evidence to support associations with body mass index, low-density lipoprotein cholesterol, total serum cholesterol, serum iron, or serum insulin-like growth factor-1 (Supplementary Table 5). There was also little evidence to support an association of genetically-proxied systolic blood pressure with colorectal cancer risk (OR per 1mmHg lower SBP: 1.00, 95% CI 0.99-1.01; P = 0.50), suggesting a potential mechanism-specific effect of this drug target on colorectal cancer risk.

Additionally, results of analyses that iteratively removed one SNP at a time from the instrument and recalculated the overall Mendelian randomization estimate were consistent, suggesting that associations were not being driven through individual influential SNPs (Supplementary Table 6).

There was little evidence that genetically-proxied ACE inhibition was associated with risk of the other cancer sites examined (Table 2).

*Genetically-proxied ADRB1 inhibition and cancer risk*

There was little evidence that genetically-proxied ADRB1 inhibition was associated with overall risk of breast, colorectal, lung, or prostate cancer (Table 3). In lung cancer subtype-stratified analyses, there was evidence to suggest an association of genetically-proxied ADRB1 inhibition with lower risk of small cell lung carcinoma (OR equivalent to 1 mmHg lower SBP: 0.87, 95% CI 0.79-0.96; P = 0.008). Colocalisation analysis suggested that ADRB1 and small cell lung carcinoma were unlikely to share a causal variant within the ADRB1 locus (1.5% posterior probability of a shared causal variant) (Supplementary Table 7, Figures 3-4). Findings for overall and subtype-specific cancer risk did not differ markedly when using an instrument for ADRB1 inhibition constructed from a GWAS unadjusted for BMI (Supplementary Table 8).

*Genetically-proxied NCC inhibition and cancer risk*
There was little evidence that genetically-proxied NCC inhibition was associated with overall risk of breast, colorectal, lung, or prostate cancer (Table 4). In oestrogen receptor-stratified breast cancer analyses, there was evidence that NCC inhibition was associated with an increased risk of ER- breast cancer (OR equivalent to 1 mmHg lower SBP: 1.20, 95% CI 1.02-1.40; \(P = 0.03\)). Colocalisation analysis provided little support for NCC and ER- breast cancer association sharing a causal variant within the \(SLC12A3\) locus (5.6% posterior probability of a shared causal variant) (Supplementary Table 9, Figures 5-6).

Replication analysis in Europeans and exploratory analysis in East Asians

Findings for genetically-proxied ACE inhibition and colorectal cancer risk were replicated in an independent sample of 1,571 colorectal cancer cases and 120,006 controls of European ancestry in the Finngen consortium (1.40, 95% CI 1.02-1.92; \(P = 0.035\)). In analyses of 23,572 colorectal cancer cases and 48,700 controls of East Asian descent, there was little evidence of association of genetically-proxied ACE inhibition and colorectal cancer risk (OR 0.97, 95% CI 0.88-1.07; \(P = 0.59\)).

Colon gene expression analysis

In transcriptome-wide analyses, genetically-proxied serum ACE inhibition was most strongly associated with \(ACE\) expression levels in the colon (\(P = 2.29 \times 10^{-31}\)). Genetically-proxied \(ACE\) expression in the colon was associated with increased odds of colorectal cancer (OR per SD increase in expression: 1.02, 95% CI 1.00-1.04; \(P = 0.01\)). However, colocalisation analysis suggested that colon \(ACE\) expression and colorectal cancer risk were unlikely to share a causal variant within the \(ACE\) locus (7.6% posterior probability of a shared causal variant) (Supplementary Table 10, Figures 7-8). Genetically-proxied serum ACE inhibition was also associated with expression levels of \(CYB561\) (\(P = 8.28 \times 10^{-11}\)) and \(FTSJ3\) (\(P = 2.95 \times 10^{-10}\)) in the colon after correction for multiple testing. \(ACE\), \(CYB561\), and \(FTSJ3\) are neighbouring genes on chromosome 17 suggesting that associations between the ACE wGRS and \(CYB561\) and \(FTSJ3\) could be driven through their co-
expression. Genetically-proxied \textit{CYB561} expression in the colon was associated with increased odds of colorectal cancer (OR per SD increase in expression: 1.06, 95% CI 1.02-1.10; \( P = 0.005 \)). However, multivariable Mendelian randomization analysis examining the association of genetically-proxied ACE inhibition with colorectal cancer risk adjusting for \textit{CYB561} expression in the colon was consistent with univariable analyses (OR 1.13, 95% CI: 0.96-1.32; \( P = 0.14 \)). Genetically-proxied \textit{FTSJ3} expression in the colon was not associated with odds of colorectal cancer (OR per SD increase in expression: 1.00, 95% CI 0.98-1.03; \( P = 0.77 \)).

In gene-set enrichment analysis of genes whose expression was associated with genetically-proxied serum ACE inhibition (\( P < 5.0 \times 10^{-3} \)), there was evidence for enrichment of expression of genes relating to memory CD8 T cells (as compared to effector CD8 T cells) in the immunologic signatures database (GSE10239) (\( P = 1.35 \times 10^{-6} \)) but little evidence for expression of other gene-sets or pathways after correction for multiple testing.

In co-expression network analysis, 30 distinct modules were defined. \textit{ACE} was in the black module along with another 659 genes. This module was correlated with the ACE wGRS (\( r = -0.11; P = 0.03 \)). Gene-set enrichment analysis of genes located in the black module showed evidence of enrichment in susceptibility genes for colorectal cancer (\( P = 1.00 \times 10^{-3} \)).

Complete findings from transcriptome-wide Mendelian randomization and gene-set enrichment analyses, along with genes from the black module from co-expression network analysis are presented in \textit{Supplementary Tables 11-14}.

\textbf{Discussion}

In this Mendelian randomization analysis of up to 266,018 cancer cases and 291,224 controls, genetically-proxied long-term ACE inhibition was associated with an increased risk of colorectal cancer. This association was restricted to cancer of the colon, with similar associations across the proximal and distal colon. There was little evidence to support associations of genetically-proxied
ACE inhibition with risk of other cancers. Genetically-proxied ADRB1 and NCC inhibition were not associated with risk of breast, colorectal, lung or prostate cancer.

Our findings for genetically-proxied ACE inhibition and colorectal cancer risk are not consistent with some previous conventional observational analyses. A meta-analysis of seven observational studies reported a protective association of ACE inhibitor use with colorectal cancer risk (OR 0.81 95% CI 0.70-0.92), though with substantial heterogeneity across studies ($I^2=71.1\%$)[65]. Interpretation of these findings is complicated by variable use of prevalent drug users, heterogenous comparator groups (both active controls and non-drug users), and the potential for immortal time bias across most included studies. Further, this meta-analysis did not include data from an earlier large Danish population-based case-control analysis with 15,560 colorectal cancer cases and 62,525 controls which reported an increased risk of colorectal cancer (OR 1.30, 95% CI 1.22-1.39) among long-term users of ACE inhibitors ($\geq 1,000$ daily doses within 5 years of study entry), as compared to never-users[7].

The potential mechanisms underpinning an association between genetically-proxied ACE inhibition and colorectal cancer risk are unclear. ACE is a multi-faceted enzyme, capable of cleaving several different peptide substrates with potential roles in carcinogenesis[66]. Along with ACE inhibition leading to an accumulation of bradykinin and substance P, both potential inducers of tumour proliferation, ACE inhibition can also lead to an increase in Ac-SDKP, an endogenous anti-fibrotic peptide which is capable of inducing angiogenesis[67]. The observed restriction of an association of genetically-proxied ACE inhibition with risk of colon, but not rectal, cancer is consistent with evidence that mRNA and protein levels of ACE are enriched in the colon but not in rectal tissue[68].

There was limited evidence of association of genetically-proxied ACE inhibition with distinct gene expression profiles in transcriptome-wide analyses. However, gene-set enrichment analysis of these findings suggested enriched expression of genes involved in immunological pathways relating to memory CD8 T cells and co-expression network analysis identified ACE expression in a cluster of co-expressed genes enriched for colorectal cancer risk susceptibility genes (e.g. $LAMA5$, $PNKD$, $TOX2$, $PLEKHG6$)[69]. These findings suggest potential future avenues of exploration to uncover mechanistic pathways linking ACE with colorectal cancer risk.
Meta-analyses of randomised trials have not reported increased rates of cancer among ACE inhibitor users, though these analyses have not reported findings separately for colorectal cancer[3, 70]. Potential discrepancies in findings for colorectal cancer between this Mendelian randomization analysis and previous clinical trials could reflect the relatively short duration of these trials (median follow-up of 3.5 years) given long induction periods of colorectal cancer. For example, the “adenocarcinoma sequence” proposes that transformation of normal colorectal epithelium to an adenoma and ultimately to invasive and metastatic cancer may occur over the course of several decades[71, 72]. Consistent with this long induction period, in randomised controlled trials examining the chemopreventive effect of aspirin on colorectal cancer risk, protective effects of aspirin are not seen until seven years after initiation of treatment, with clear risk reductions becoming apparent only after ten years of follow-up [73]. Alternatively, it may be possible that an effect of ACE inhibition on cancer is restricted solely to the earliest stages of the adenoma-carcinoma sequence and therefore may not influence cancer risk among largely middle-aged participants of clinical trials if dysplasia is already present. Finally, it is possible that lower levels of circulating ACE concentrations may influence colorectal cancer risk only during a particular critical or sensitive period of the life-course (e.g. in childhood or adolescence), given some evidence to suggest a potential role of early-life factors in colorectal carcinogenesis[74].

Our largely null findings for genetically-proxied ACE inhibition and risk of breast, lung, and prostate cancer risk are not consistent with some previous observational reports that compared ACE inhibitor users to non-users or to users of β blockers or thiazide diuretics[7, 9, 15]. However, our findings for genetically-proxied ACE inhibition are in agreement with those from short-term randomised controlled trials for these site-specific cancers and suggest that long-term use of these drugs may not influence cancer risk, though we cannot rule out small effects from their long-term use[3]. Likewise, our findings for ADRB1 and NCC are in agreement with short-term trial data reporting no association of β blockers and thiazide diuretics use with overall cancer risk[2].

Strengths of this analysis include the use of cis-acting variants in genes encoding antihypertensive drug targets to proxy inhibition of these targets which should minimise confounding, the employment
of various sensitivity analyses to rigorously assess for violations of Mendelian randomization assumptions, and the use of a summary-data Mendelian randomization approach which permitted us to leverage large-scale genetic data from several cancer GWAS consortia, enhancing statistical power and precision of causal estimates. As with prior Mendelian randomization analyses of antihypertensive drug targets that used similar approaches to instrument construction to our analysis, the general concordance of estimates of the effect of these instruments on cardio-metabolic endpoints with those reported in prior clinical trials for these medications supports the plausibility of these instruments[75, 76]. Finally, the use of germline genetic variants as proxies for antihypertensive drug targets facilitated evaluation of the effect of the long-term inhibition of these targets, which may be more representative of the typically decades-long use of antihypertensive therapy as compared to periods of medication use typically examined in conventional observational studies and randomised trials.

There are several limitations to these analyses. First, Mendelian randomization analyses are restricted to examining on-target (i.e. target-mediated) effects of therapeutic interventions. Second, statistical power was likely limited in some analyses of less common cancer subtypes. Limited statistical power in analyses of genetically-proxied ACE inhibition and colorectal cancer risk in East Asians (instrumented by rs4343) may also have accounted for the lack of association between these traits within this population. Third, while these analyses did not account for previously reported associations of genetically-proxied elevated systolic blood pressure with antihypertensive medication use within the colorectal cancer datasets analysed, such correction would be expected to strengthen, rather than attenuate, findings presented in this study[77]. Fourth, though mixed-ancestry GWAS were used to construct instruments for serum ACE concentrations, effect allele frequencies for variants used in this instrument were similar across European and Latin American ancestry participants, suggesting that Mendelian randomization findings were unlikely to be influenced by confounding through residual population stratification. Fifth, effect estimates presented make the additional assumptions of linearity and the absence of gene-environment and gene-gene interactions. Sixth, our genetically-proxied ADRB1 findings are of greater relevance to second generation β
blockers (e.g. atenolol and metoprolol) which selectively inhibit ADRB1 as compared to first
generation β blockers (e.g. propranolol and nadolol) which equally inhibit ADRB1 and ADRB2[78].
Future Mendelian randomization analyses examining the potential effects of long-term first generation
β blocker use incorporating both ADRB1 and ADRB2 variants is warranted. Seventh, we cannot rule
out findings presented being influenced by canalization (i.e. compensatory processes being generated
during development that counter the phenotypic impact of genetic variants being used as instruments).
Finally, while various sensitivity analyses were performed to examine exchangeability and exclusion
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Colorectal cancer is the third most common cause of cancer globally [79]. Given the prevalence of
ACE inhibitor use in high- and middle-income countries and growing use in low-income countries,
and the often long-term nature of antihypertensive therapy, these findings, if replicated in subsequent
clinical trials, may have important implications for choice of antihypertensive therapy[4]. Importantly,
given that hypertension is more prevalent among those who are overweight or obese (risk factors for
colorectal cancer), these findings suggest that long-term use of this medication could increase
colorectal cancer risk among populations who are already at elevated risk of this disease. Further
work is warranted to unravel molecular mechanisms underpinning the association of ACE with
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prognosis among colorectal cancer patients. Finally, findings from this analysis should be
“triangulated” by employing other epidemiological designs with orthogonal (i.e. non-overlapping)
sources of bias to each other to further evaluate the association of ACE inhibition and colorectal
cancer risk[80].

Conclusion

Our Mendelian randomization analyses suggest that genetically-proxied long-term inhibition of ACE
may increase risk of colorectal cancer. Evaluation of ACE inhibitor use in randomised controlled
trials with sufficient follow-up data can inform on the long-term safety of these medications. Our findings provide human genetic support to short-term randomised trials that long-term use of β blockers and thiazide diuretics may not influence risk of common cancers.
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Table 1. Characteristics of systolic blood pressure lowering genetic variants in *ACE*, *ADRB1*, and *SLC12A3*

| Target | Effect allele/Non-Effect allele | Effect Allele Frequency | Effect (SE) | P-value |
|--------|---------------------------------|-------------------------|-------------|---------|
| **ACE** |                                |                         |             |         |
| rs4343 | A/G                             | 0.45                    | -0.63 (0.02) | 1.53 x 10⁻²¹³ |
| rs12452187 | A/G                      | 0.60                    | -0.23 (0.02) | 2.53 x 10⁻²¹ |
| rs79480822 | C/T                     | 0.93                    | -0.55 (0.05) | 6.37 x 10⁻²⁴ |
| rs3730025 | G/A                         | 0.01                    | -0.80 (0.09) | 4.32 x 10⁻¹⁹ |
| rs11655956 | C/G                     | 0.08                    | -0.35 (0.04) | 1.06 x 10⁻¹⁵ |
| rs118121655 | G/A                  | 0.96                    | -0.54 (0.07) | 3.10 x 10⁻¹³ |
| rs4365 | G/A                             | 0.97                    | -0.58 (0.08) | 7.06 x 10⁻¹² |
| rs4968771 | G/A                         | 0.08                    | -0.22 (0.03) | 1.78 x 10⁻¹¹ |
| rs12150648 | G/A                     | 0.96                    | -0.39 (0.06) | 1.88 x 10⁻¹⁰ |
| rs80311894 | T/G                     | 0.97                    | -0.46 (0.07) | 2.60 x 10⁻¹⁰ |
| rs118138685 | C/G                 | 0.04                    | -0.40 (0.07) | 2.44 x 10⁻⁹  |
| rs13342595 | C/T                    | 0.23                    | -0.14 (0.02) | 2.48 x 10⁻⁷  |
| rs28656895 | T/C                    | 0.23                    | -0.14 (0.02) | 3.77 x 10⁻⁷  |
| rs4968780 | C/A                             | 0.05                    | -0.28 (0.05) | 1.86 x 10⁻⁸  |

| **ADRB1** |                                |                         |             |         |
| rs1801253 | G/C                             | 0.23                    | -0.41 (0.03) | 8.07 x 10⁻⁴¹ |
| rs11196549 | G/A                         | 0.96                    | -0.62 (0.07) | 2.53 x 10⁻¹⁹ |
| rs4918889 | G/C                             | 0.17                    | -0.30 (0.04) | 7.53 x 10⁻¹⁸ |
| rs460718  | A/G                             | 0.33                    | -0.24 (0.03) | 2.21 x 10⁻¹⁷ |
| rs11196597 | G/A                         | 0.86                    | -0.27 (0.04) | 3.07 x 10⁻¹² |
| rs143854972 | G/A                     | 0.94                    | -0.39 (0.06) | 4.35 x 10⁻¹¹ |
| rs17875473 | C/T                          | 0.91                    | -0.28 (0.05) | 9.04 x 10⁻⁹  |
| rs10787510 | A/G                             | 0.48                    | -0.15 (0.03) | 2.01 x 10⁻⁸  |

| **NCC** |                                |                         |             |         |
| rs35797045 | A/C                          | 0.05                    | -0.35 (0.06) | 4.85 x 10⁻⁸  |

Effect (SE) represents change in serum ACE concentrations per additional copy of the effect allele for ACE analysis and change in systolic blood pressure per additional copy of the effect allele for ADRB1 and NCC analyses. In analyses of genetically-proxied ACE inhibition and colorectal cancer risk, one SNP (rs8064760) was not available in the colorectal cancer dataset. Two SNPs associated with systolic blood pressure used to proxy ACE inhibition in sensitivity analyses were: rs8077276 (effect allele/non-effect allele: A/G, effect (se): -0.27 (0.03), effect allele frequency: 0.62; P-value: 4.47 x 10⁻²²) and rs28656895 (effect allele/non-effect allele: T/C, effect (se): -0.19 (0.03), effect allele frequency: 0.23; P-value: 3.37 x 10⁻⁹).
Table 2. Association between genetically-proxied ACE inhibition and risk of overall and subtype-specific breast, colorectal, prostate, and lung cancer risk

| Outcome                      | N (cases, controls) | OR (95% CI)      | P-value |
|------------------------------|---------------------|------------------|---------|
| Breast cancer                | 122,977; 105,974    | 0.98 (0.94-1.02) | 0.35    |
| ER+ Breast cancer            | 69,501; 105,974     | 0.99 (0.94-1.04) | 0.76    |
| ER- Breast cancer            | 21,468; 105,974     | 0.97 (0.90-1.05) | 0.47    |
| Colorectal cancer            | 58,221; 67,694      | 1.13 (1.06-1.22) | 3.6 x 10^-4 |
| Colon cancer                 | 32,002; 64,159      | 1.18 (1.07-1.31) | 9.7 x 10^-4 |
| Rectal cancer                | 16,212; 64,159      | 1.07 (0.97-1.18) | 0.16    |
| Lung cancer                  | 29,863; 55,586      | 1.01 (0.92-1.10) | 0.93    |
| Lung adenocarcinoma          | 11,245; 54,619      | 1.02 (0.91-1.15) | 0.70    |
| Small cell lung carcinoma    | 2,791; 20,580       | 0.96 (0.76-1.20) | 0.71    |
| Squamous cell lung cancer    | 7,704; 54,763       | 0.97 (0.81-1.16) | 0.73    |
| Prostate cancer              | 79,148; 61,106      | 1.06 (0.99-1.13) | 0.08    |
| Advanced prostate cancer     | 15,167; 58,308      | 1.05 (0.94-1.17) | 0.37    |

OR represents the exponential change in odds of cancer per genetically proxied inhibition of ACE equivalent to a 1 mmHg decrease in systolic blood pressure.
Table 3. Association between genetically-proxied ADRB1 inhibition and risk of overall and subtype-specific breast, colorectal, prostate, and lung cancer risk

| Outcome                  | N (cases, controls) | OR (95% CI)       | P-value |
|--------------------------|---------------------|-------------------|---------|
| Breast cancer            | 122,977; 105,974    | 1.01 (0.99-1.04)  | 0.38    |
| ER+ Breast cancer        | 69,501; 105,974     | 1.01 (0.98-1.04)  | 0.44    |
| ER- Breast cancer        | 21,468; 105,974     | 0.98 (0.94-1.02)  | 0.38    |
| Colorectal cancer        | 58,221; 67,694      | 0.98 (0.96-1.01)  | 0.31    |
| Colon cancer             | 32,002; 64,159      | 0.99 (0.95-1.03)  | 0.63    |
| Rectal cancer            | 16,212; 64,159      | 1.00 (0.95-1.04)  | 0.84    |
| Lung cancer              | 29,863; 55,586      | 1.01 (0.96-1.07)  | 0.64    |
| Lung adenocarcinoma      | 11,245; 54,619      | 0.98 (0.91-1.04)  | 0.48    |
| Small cell lung carcinoma| 2,791; 20,580       | 0.87 (0.79-0.96)  | 0.008   |
| Squamous cell lung cancer| 7,704; 54,763       | 0.98 (0.91-1.06)  | 0.67    |
| Prostate cancer          | 79,148; 61,106      | 1.00 (0.96-1.03)  | 0.73    |
| Advanced prostate cancer | 15,167; 58,308      | 1.00 (0.94-1.06)  | 0.97    |

OR represents the exponential change in odds of cancer per genetically proxied inhibition of ADRB1 equivalent to a 1 mmHg decrease in systolic blood pressure.
Table 4. Association between genetically-proxied NCC inhibition and risk of overall and subtype-specific breast, colorectal, prostate, and lung cancer risk

| Outcome                        | N (cases, controls) | OR (95% CI) | P-value |
|--------------------------------|---------------------|-------------|---------|
| Breast cancer                  | 122,977; 105,974    | 1.08 (0.99-1.18) | 0.08    |
| ER+ Breast cancer              | 69,501; 105,974     | 1.06 (0.95-1.18) | 0.28    |
| ER- Breast cancer              | 21,468; 105,974     | 1.20 (1.02-1.40) | 0.03    |
| Colorectal cancer              | 58,221; 67,694      | 1.09 (0.96-1.23) | 0.19    |
| Colon cancer                   | 32,002; 64,159      | 1.03 (0.89-1.19) | 0.69    |
| Rectal cancer                  | 16,212; 64,159      | 1.13 (0.94-1.36) | 0.20    |
| Lung cancer                    | 29,863; 55,586      | 1.09 (0.89-1.33) | 0.38    |
| Lung adenocarcinoma            | 11,245; 54,619      | 1.01 (0.81-1.26) | 0.95    |
| Small cell lung carcinoma      | 2,791; 20,580       | 1.12 (0.76-1.53) | 0.57    |
| Squamous cell lung cancer      | 7,704; 54,763       | 1.00 (0.78-1.29) | 0.99    |
| Prostate cancer                | 79,148; 61,106      | 1.08 (0.96-1.19) | 0.18    |
| Advanced prostate cancer       | 15,167; 58,308      | 1.05 (0.86-1.28) | 0.63    |

OR represents the exponential change in odds of cancer per genetically proxied inhibition of NCC equivalent to a 1 mmHg decrease in systolic blood pressure.
Serum ACE concentrations

Plotted SNPs

rs4343

\( r^2 \)

\( r^2 \)

\( TANC2 \)

\( ACE \)

\( KCNH6 \)

\( DCAF7 \)

\( CYB561 \)

Position on chr17 (Mb)
Colorectal cancer risk

-\log_{10}(p\text{-value})

Recombination rate (cM/Mb)

rs4343

Position on chr17 (Mb)

\textbf{TANC2}→

\textbf{ACE}→

\textbf{KCNH6}→

\textbf{DCAF7}→

\textbf{CYB561}←
Small cell lung carcinoma risk

Position on chr10 (Mb)
Systolic blood pressure

Plotted SNPs

-10

log10(p-value)

rs35797045

NUP93

SLC12A3

HERPUD1

CETP

MIR138-2

MIR6863

Position on chr16 (Mb)

Recombination rate (cM/Mb)

r²

0.2

0.4

0.6

0.8

1.0

0

20

40

60

80

100

0

2

4

6

8

10
ER− breast cancer risk

--log_{10}(p-value)

NUP93 → SLC12A3 → HERPUD1 → CETP → MIR138−2 → MIR6863

rs35797045

Position on chr16 (Mb)

Recombination rate (cM/Mb)

rs35797045
Colon ACE gene expression

-\log_{10}(p\text{-value}) vs. Recombination rate (cM/Mb)

Plotted SNPs:
- rs4292

Genes:
- MIR548W
- CYB561
- KCNH6
- TACO1
- LIMD2
- CCDC47
- TANC2
- ACE
- DCAF7
- MAP3K3
- LOC729683
- STRADA
- DDX42

Position on chr17 (Mb):
- 61.3
- 61.4
- 61.5
- 61.6
- 61.7
- 61.8
