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Citation

Yarmolinsky, J., Lee, M. A., Lau, E., Moratalla-Navarro, F., Vincent, E. E., Li-Gao, R. F., ... Tzoulaki, I. (2025). Proteogenomic and observational evidence implicate ANGPTL4 as a potential therapeutic target for colorectal cancer prevention. *Journal Of The National Cancer Institute*. doi:10.1093/jnci/djaf137

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Note: To cite this publication please use the final published version (if applicable).

Proteogenomic and observational evidence implicate ANGPTL4 as a potential therapeutic target for colorectal cancer prevention

James Yarmolinsky , PhD^{*1}, Matthew A. Lee , PhD², Evelyn Lau , PhD³, Ferran Moratalla-Navarro , PhD^{4,5,6,7}, Emma E. Vincent , PhD^{8,9,10}, Ruifang Li-Gao , PhD¹¹, Patrick C.N. Rensen , PhD^{12,13}, Ko Willems van Dijk , PhD^{12,13,14}, Kostas K. Tsilidis , PhD^{1,15}, Apiwat Sangphukieo , PhD¹⁶, Elmira Ebrahimi , MSc¹⁶, Jochen Hampe , MD, PhD¹⁷, Loïc Le Marchand , MD, PhD¹⁸, Franzel J.B. van Duijnhoven , PhD¹⁹, Kala Visvanathan , MD²⁰, Michael O. Woods , PhD²¹, Marcela Guevara , MD, PhD^{22,23,24}, Sabina Sieri , PhD²⁵, Giovanna Masala , MD²⁶, Keren Papier , PhD²⁷, Shama Virani , PhD¹⁶, Tom Dudding , PhD²⁸, Abbas Dehghan , MD, PhD^{1,29}, Alexander G. Smith , PhD¹, Dennis Wang , PhD^{3,30}, Victor Moreno , PhD^{4,5,6,7}, Marc J. Gunter, PhD^{1,2}, Ioanna Tzoulaki , PhD^{1,29,31}

¹Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom

²Nutrition and Metabolism Branch (NME), International Agency for Research on Cancer, Lyon, France

³Institute for Human Development and Potential, Agency for Science, Technology and Research (A*STAR), Singapore, Republic of Singapore

⁴Unit of Biomarkers and Susceptibility (UBS), Oncology Data Analytics Program (ODAP), Catalan Institute of Oncology (ICO), L'Hospitalet del Llobregat, Barcelona, Spain

⁵ONCOBELL Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain

⁶Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain

⁷Department of Clinical Sciences and Universitat de Barcelona Institute of Complex Systems (UBICS), Faculty of Medicine, University of Barcelona, Barcelona, Spain

⁸MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom

⁹Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom

¹⁰Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom

¹¹Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

¹²Division of Endocrinology, Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands

¹³Eindhoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

¹⁴Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

¹⁵Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece

¹⁶Genomic Epidemiology Branch, International Agency for Research on Cancer, Lyon, France

¹⁷Department of Medicine I, University Hospital Dresden, Technische Universität Dresden (TU Dresden), Dresden, Germany

¹⁸Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI, United States

¹⁹Division of Human Nutrition and Health, Wageningen University & Research, Wageningen, The Netherlands

²⁰Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

²¹Discipline of Genetics, Memorial University of Newfoundland, St John's, NL, Canada

²²Instituto de Salud Pública y Laboral de Navarra, Pamplona, Spain

²³Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

²⁴Navarra Institute for Health Research (IdiSNA), Pamplona, Spain

²⁵Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

²⁶Clinical Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Florence, Italy

²⁷Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom

²⁸Bristol Dental School, University of Bristol, Bristol, United Kingdom

²⁹Dementia Research Institute, Imperial College London, London, United Kingdom

³⁰National Heart and Lung Institute, Imperial College London, London, United Kingdom

³¹Systems Biology, Biomedical Research Foundation Academy of Athens, Athens, Greece

*Corresponding author: James Yarmolinsky, PhD, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, 90 Wood Lane, London W12 0BZ, United Kingdom (j.yarmolinsky@imperial.ac.uk)

Author Contributions: M.J. Gunter and I. Tzoulaki contributed equally to this work.

Abstract

Background: The role of lipid-perturbing medications in cancer risk is unclear.

Methods: We employed cis-Mendelian randomization and colocalization to evaluate the role of 5 lipid-perturbing drug targets (ANGPTL3, ANGPTL4, APOC3, CETP, and PCSK9) in risk of 5 cancers (breast, colorectal, head and neck, ovarian, and prostate). We triangulated findings using pre-diagnostic protein measures in prospective analyses in EPIC (977 colorectal cancer cases, 4080 sub-cohort members) and the UK Biobank (860 colorectal cancer cases, 50 177 controls). To gain mechanistic insight into the role of ANGPTL4 in carcinogenesis, we examined the impact of the ANGPTL4 p. E40K loss-of-function variant on differential gene expression

Received: January 14, 2025. Revised: May 06, 2025. Accepted: May 27, 2025

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in normal colon tissue in BarcUva-Seq. Finally, we evaluated the association of colon tumor ANGPTL4 expression with cancer-specific mortality in TCGA.

Results: In analysis of 78 473 cases and 107 143 controls, genetically proxied circulating ANGPTL4 inhibition was associated with reduced colorectal cancer risk ($OR_{SD\ decrease} = 0.76$, 95% confidence interval [CI] = 0.66 to 0.89, $P = 5.52 \times 10^{-4}$, $PP_{colocalization} = 0.83$). This association was replicated using pre-diagnostic circulating ANGPTL4 concentrations in EPIC (hazard ratio $[HR]_{log10\ decrease} = 0.91$, 95% CI = 0.84 to 0.98, $P = .01$) and the UK Biobank ($HR_{SD\ decrease} = 0.93$, 95% CI = 0.86 to 0.99, $P = .03$). In gene-set enrichment analysis of differential gene expression in 445 colon tissue samples, ANGPTL4 loss-of-function down-regulated several cancer-related biological pathways ($P_{FDR} < .05$), including those involved in cellular proliferation, epithelial-to-mesenchymal transition, and bile acid metabolism. In analysis of 465 colon cancer patients, lower ANGPTL4 tumor expression was associated with reduced colorectal cancer-specific mortality risk ($HR_{log2\ decrease} = 0.66$, 95% CI = 0.50 to 0.87, $P = 2.92 \times 10^{-3}$).

Conclusions: Our integrative proteogenomic and observational analyses suggest a potential protective role of lower circulating ANGPTL4 concentrations in colorectal cancer risk. These findings support further evaluation of ANGPTL4 as a therapeutic target for colorectal cancer prevention.

Introduction

Lipids perform various essential physiological functions including providing an energy reserve, serving as structural components of cell membranes, and participating in cellular signaling.¹ It is well established that select lipid parameters (eg, LDL cholesterol) contribute to atherosclerotic cardiovascular disease (ASCVD). Along with their role in ASCVD, preclinical studies have suggested that lipids may also influence carcinogenesis through several mechanisms including those related to insulin resistance, inflammation, and oxidative stress.² Reprogramming of lipid metabolism also plays a critical role in promoting tumorigenesis and is considered an emerging hallmark of cancer.^{3,4} For example, cancer cells must harness lipid metabolism to support cell division, adapt to stress, and enable metastatic dissemination.⁵⁻⁷ In addition, lipid metabolic reprogramming can remodel the tumor microenvironment by influencing the recruitment, survival, and function of immune cells.⁸

Consistent with a role of circulating lipids in cancer development, preclinical and epidemiological studies have suggested that several lipid-perturbing medications may lower cancer risk.⁹⁻¹³ For example, knockdown of ANGPTL3, the target of the lipid-lowering therapy evinacumab, has been shown to suppress proliferation, migration, and invasion in several cancer cell lines.¹⁴⁻¹⁶ In addition, PCSK9 inhibition using siRNA, gene knock-out, or anti-PCSK9 vaccination promotes apoptosis in in vitro cancer models.¹³ Observational epidemiological studies have also reported that long-term statin users have lower rates of site-specific cancer as compared with non-users.^{12,17-21}

These findings collectively suggest the potential for repurposing approved and/or emerging lipid-perturbing cardiovascular disease (CVD) medications for cancer prevention. However, in the absence of randomized clinical trial data, the causal nature of these medications in cancer onset, and thus their suitability as intervention targets, is unclear. This is because of the uncertain relevance of preclinical disease models to humans and the susceptibility of conventional observational analyses to residual confounding and reverse causation, undermining confident causal inference.²²⁻²⁴

Here, we leveraged 4 complementary epidemiological approaches to triangulate evidence on the potential causal role of lipid-perturbing drug targets in cancer risk. We employed drug-target Mendelian randomization (MR) to systematically evaluate the effect of 5 approved or emerging lipid-perturbing drug targets for CVD (APOC3, ANGPTL3, ANGPTL4, CETP, and PCSK9) on risk of 5 cancers (breast, colorectal, head and neck, ovarian, and prostate). This approach leverages the natural randomization of germline genetic variants at meiosis and can

minimize conventional epidemiological issues of confounding and reverse causation. We then examined the association of pre-diagnostic direct measures of circulating protein targets and cancer risk in prospective analyses in the European Prospective Investigation into Cancer and Nutrition (EPIC) and the UK Biobank. To gain mechanistic insight into the role of ANGPTL4 in carcinogenesis, we explored the impact of ANGPTL4 loss-of-function on differential gene expression in normal colon tissue samples in the University of Barcelona and the University of Virginia Genotyping and RNA Sequencing (BarcUva-Seq) project. Finally, to explore whether ANGPTL4 is involved in cancer progression, we evaluated the association of ANGPTL4 gene expression in colon tumor tissue with all-cause mortality in The Cancer Genome Atlas (TCGA).

Methods

A step-by-step overview of the analytical stages of this work is presented in Figure 1.

Study populations

We selected cancer outcomes where there was prior evidence from Mendelian randomization studies to suggest a role of circulating lipids (ie, breast, colorectal) or lipid-perturbing drug targets (ie, head and neck, ovarian, prostate) in their etiology.²⁵⁻³¹

For drug-target MR analyses, summary genetic association estimates for overall and histological or anatomical subtype-stratified cancer risk were obtained from analyses of up to 319 661 cancer cases and 348 078 controls across 5 consortia.³²⁻³⁶ For genetic instrument validation analyses, summary genetic association data on circulating LDL cholesterol, HDL cholesterol, and triglycerides were obtained from analyses of ~1.3 million participants in the Global Lipids Genetics Consortium (GLGC) analysis.³⁷ These analyses were restricted to participants of European ancestry. 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.

For observational analyses using direct measures of protein drug targets, we used data from the EPIC cohort study.³⁸ We limited analyses to 10 261 individuals recruited into a multi-endpoint case-cohort within EPIC, of whom 6876 had incident cancer, including 977 colorectal cancer cases (658 colon cancer, 319 rectal cancer).

For transcriptomic analyses examining the effect of ANGPTL4 loss-of-function on differential gene expression, we obtained combined germline genotype and RNA sequencing data from 445

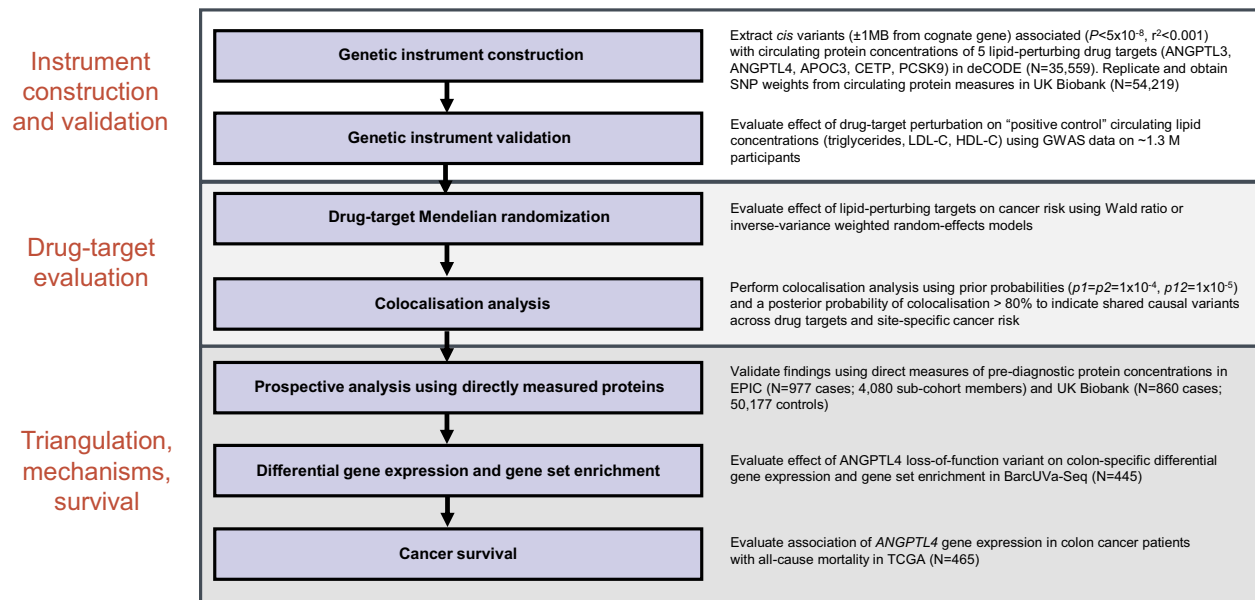


Figure 1. EPIC = European Prospective Investigation into Cancer and Nutrition; GWAS = genome-wide association study; TCGA = The Cancer Genome Atlas.

normal (ie, non-neoplastic) colon tissue samples in BarcUVA-Seq.³⁹

For analyses examining the association of tumor gene expression with all-cause mortality, we obtained gene expression (RNA-Seq), demographic, and clinicopathological data from 465 colon adenocarcinoma (TCGA-COAD) cases in TCGA.⁴⁰

Genetic instrument construction

Genetic instruments for drug targets were constructed using a 2-stage strategy to minimize bias from "Winner's curse" (further information on instrument construction is presented in the [Supplementary Methods](#)). In brief, genetic instruments were constructed from genome-wide significant ($P < 5 \times 10^{-8}$) and independent ($LD\ r^2 < 0.001$) single-nucleotide polymorphisms (SNPs) in or within 1MB from the gene encoding the relevant protein (N = 35 559 individuals of Icelandic ancestry) then tested for replication in an independent GWAS of 54 219 individuals of primarily white British ancestry in the UK Biobank.^{41,42} For SNPs that replicated ($P < .05$) and were directionally consistent, SNP weights were obtained from UK Biobank analyses. Genetic instruments for lipid-perturbing drug targets were constructed using circulating plasma protein levels (ie, as compared with downstream biomarkers of drug target perturbation) to increase instrument strength given that circulating plasma protein levels are expected to be more proximal to *cis*-acting variants influencing these traits.

Drug-target Mendelian randomization primary and sensitivity analyses

Drug-target Mendelian randomization uses germline genetic variants as instrumental variables to estimate on-target effects of perturbation of drug targets, under specific assumptions (described in the [Supplementary Methods](#)).

For drug targets instrumented by a single SNP, the Wald ratio was used to generate effect estimates and the delta method was used to approximate standard errors. For drug targets instrumented by 2 or more SNPs, inverse-variance weighted (IVW) random-effects models were used to estimate causal effects.⁴³

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. We evaluated the "exclusion restriction" assumption by performing various sensitivity analyses. First, we validated our instruments by evaluating the effect of genetically proxied drug targets on downstream biomarkers influenced (ie, for approved drugs) or presumed to be influenced (ie, for emerging drugs) by the target as "positive control" analyses (ie, triglyceride concentrations for APOC3, ANGPTL3, ANGPTL4; HDL cholesterol concentrations for CETP; LDL cholesterol concentrations for PCSK9).^{25,44-47} Second, colocalization was performed to evaluate whether drug targets and both "positive control" lipid measures and cancer outcomes showing evidence of association in MR analyses (Bonferroni-corrected $P < 6.17 \times 10^{-4}$) were likely to share the same causal variant at a given locus. We used a posterior probability of colocalization ($PP_{\text{colocalization}} > 0.80$) to support colocalization of drug targets and disease outcomes. Third, for analyses examining the association of circulating triglycerides with colorectal cancer risk, we employed 3 complementary "pleiotropy-robust" models: MR-Egger regression, weighted median estimation, and weighted mode estimation.⁴⁸⁻⁵⁰

To account for multiple testing across drug-target MR analyses, a Bonferroni correction was used ($P < 6.17 \times 10^{-4}$) (false-positive rate = 0.05/81 statistical tests).

Association of pre-diagnostic ANGPTL4 concentrations and colorectal cancer risk

In analysis of directly measured pre-diagnostic ANGPTL4 concentrations in EPIC, we employed Cox proportional hazards models with age as the time scale and considered "minimally adjusted" (ie, stratified on sex, center of origin, and age at recruitment), "lifestyle adjusted" (ie, further adjusted for body mass index, alcohol consumption, smoking, physical activity, and education level), and "dietary factor adjusted" (ie, further adjusted for total daily energy intake, total daily red meat intake, total daily processed meat intake, total daily fiber intake, and total daily calcium intake) models. Prentice weights and robust variance were used to account for the case-cohort design. We repeated analyses

stratified by sub-site (colon cancer, rectal cancer) and tested for heterogeneity by sub-site. Lag analyses were performed by repeating analyses excluding participants within the first 2 and 5 years of follow-up.

Analyses of pre-diagnostic ANGPTL4 concentrations and colorectal cancer risk in the UK Biobank cohort study used Cox proportional hazards models with age as the time scale. “Minimally adjusted” (ie, adjusted for sex, age at recruitment), “lifestyle adjusted” (ie, further adjusted for body mass index, alcohol consumption, smoking, physical activity, and education levels), and “dietary factor adjusted” (ie, further adjusted for red meat intake, processed meat intake, total daily energy intake, total daily calcium intake, and total daily fiber intake) models were employed. Analyses were repeated stratified on colorectal cancer subsite (colon cancer, rectal cancer) and “2 year” and “5 year” lag analyses were performed. Further information on cancer case definition and covariate classification across both EPIC and UK Biobank analyses is presented in the [Supplementary Methods](#).

Impact of ANGPTL4 loss-of-function on colon differential gene expression and gene set enrichment

To provide potential mechanistic insight into the effect of ANGPTL4 on precancerous molecular changes within the colon, we performed a phenome-wide association study of ANGPTL4 loss-of-function on differential gene expression in 445 normal colon tissue samples. For these analyses, we employed p. E40K (rs116843064), a variant that has been shown to abolish ANGPTL4 function.⁵¹ This variant was also used as a genetic instrument for circulating ANGPTL4 concentrations in drug-target MR analysis.

We then performed gene set enrichment analysis on genes whose expression was associated with p. E40K ($P < .05$) to identify biological pathways enriched among these genes using the Human MSigDB Collections Hallmark gene set.⁵² Gene set enrichment was performed using the fgsea R package with 1000 permutations.⁵³ Further details on RNA-Seq and genotype processing, transcriptome-wide gene expression, and loss-of-function analyses are presented in the [Supplementary Methods](#).

ANGPTL4 tumor expression and all-cause mortality in colon cancer patients

To explore if ANGPTL4 is involved in cancer prognosis, we evaluated the association of ANGPTL4 tumor expression with all-

cause mortality in 481 colon cancer patients in TCGA. Read counts were normalized using the trimmed mean of M-values (TMM) method and then transformed to log2-counts per million reads. Cox proportional hazards models were employed with adjustment for age at diagnosis, gender, race, and American Joint Committee on Cancer (AJCC) pathologic stage. The time-to-event period was defined as the number of days between the initial diagnosis date and death or last follow-up. After excluding 16 participants with missing covariate data, there were 465 colon cancer patients. We also examined the association of ANGPTL4 tumor expression with colorectal cancer-specific mortality using disease-specific survival (DSS) data defined and curated by the TCGA Clinical Data Resource.⁵⁴ We did not explore the association of ANGPTL4 expression with rectal cancer because of the limited number of events ($N = 23$) in this analysis.

This study is reported as per the STROBE-MR reporting guidelines.⁵⁵ All statistical analyses were performed using R version 4.3.1.

Results

Genetic instrument strength and validation analyses

Across the 5 drug targets, F-statistics for their instruments ranged from 306 to 3388, suggesting that genetic instruments were unlikely to suffer from weak instrument bias. Characteristics of genetic variants used to proxy drug targets are presented in [Table 1](#). Estimates of r^2 and F-statistics for each target are presented in [Table S1](#).

Findings from genetic instrument validation analyses using Mendelian randomization and colocalization were consistent with previously reported effects of approved and emerging medications on circulating lipid biomarkers reported in clinical trials ([Table S2](#)).

Genetically proxied drug target perturbation and cancer risk

In analysis of 78 473 cases and 107 143 controls, there was evidence that genetically proxied circulating ANGPTL4 inhibition was associated with a reduced risk of colorectal cancer (odds ratio [OR] per SD decrease = 0.76, 95% confidence interval [CI] = 0.66 to 0.89, $P = 5.52 \times 10^{-4}$) ([Table S3](#)). There was a high posterior probability that circulating ANGPTL4 concentrations and colorectal cancer risk shared a causal variant within the ANGPTL4

Table 1. Characteristics of genetic variants used to proxy lipid-perturbing drug targets.

Target	SNP	Effect allele/non-effect allele	Effect allele frequency	Beta (SE)	P
ANGPTL3					
ANGPTL4	rs10889352	C/T	0.35	−0.27 (0.01)	$< 5 \times 10^{-324}$
	rs116843064	A/G	0.02	−0.35 (0.02)	5.70×10^{-54}
APOC3					
	rs964184	C/G	0.88	−0.21 (0.01)	2.69×10^{-65}
	rs187929675	T/C	0.01	−0.44 (0.04)	2.78×10^{-29}
	rs141469619	A/G	0.99	−0.26 (0.04)	4.98×10^{-13}
CETP					
	rs183130	T/C	0.33	−0.32 (0.01)	5.83×10^{-136}
	rs158482	G/T	0.98	−0.31 (0.05)	1.81×10^{-9}
PCSK9					
	rs11591147	T/G	0.02	−1.12 (0.02)	$< 5 \times 10^{-324}$
	rs472495	G/T	0.36	−0.15 (0.01)	5.72×10^{-131}

Beta (SE) represents the change in circulating concentrations of the respective drug target per additional copy of the effect allele. Abbreviations: ANGPTL3 = Angiopoietin-like 3; ANGPTL4 = Angiopoietin-like 4; APOC3 = Apolipoprotein C3; CETP = Cholesteryl ester transfer protein; PCSK9 = Proprotein convertase subtilisin/kexin type 9; SNP = single-nucleotide polymorphism. Estimates were obtained from the UK Biobank for ANGPTL3, ANGPTL4, and PCSK9.

locus ($PP_{\text{colocalization}} = 0.83$). In analyses stratified on colorectal cancer subsite, there was weak evidence for differences in risk of colon cancer (OR = 0.86, 95% CI = 0.69 to 1.08, $P = .19$) and rectal cancer (OR = 0.64, 95% CI = 0.47 to 0.86, $P = 3.20 \times 10^{-3}$) ($P_{\text{het}} = 0.12$).

ANGPTL4 is a key regulator of plasma triglyceride levels, and therefore, we examined whether the association of genetically proxied ANGPTL4 inhibition was driven by reductions in circulating triglycerides. In MR analysis, we found little evidence of association of genetically proxied triglyceride concentrations with colorectal cancer risk in a primary IVW model (OR per unit decrease in log-transformed triglycerides = 1.04, 95% CI = 0.98 to 1.10; $P = .22$) or in pleiotropy-robust models (Table S4).

Genetically proxied ANGPTL4 inhibition was not associated with risk of 5 other cancers examined (FDR $P < .05$). Likewise, there was no evidence of association of genetically proxied ANGPTL3, APOC3, CETP, or PCSK9 inhibition with cancer risk (FDR $P < .05$) (Tables S5–S8). As such, subsequent analyses were restricted to ANGPTL4 and colorectal cancer and its subsites only.

Association of pre-diagnostic ANGPTL4 concentrations and colorectal cancer risk

Case-cohort analyses in EPIC included 977 incident colorectal cancer cases and 4080 subcohort members (median 15.5 year follow-up). Compared with those in the lowest quartile, participants in the highest quartile of baseline circulating ANGPTL4 concentrations had higher levels of alcohol intake and were more likely to be a current smoker and to be physically active (Table 2). In the minimally adjusted multivariable regression model, we found evidence of a protective association of lower circulating ANGPTL4 concentrations (HR per log10 decrease = 0.91, 95% CI = 0.84 to 0.98, $P = .01$), consistent with genetic analyses. Findings did not materially change when further adjusted for lifestyle factors (HR per log10 decrease = 0.92, 95% CI = 0.85 to 0.99, $P = .02$) and dietary factors (HR = 0.92, 95% CI = 0.85 to 0.99, $P = .03$). The association of circulating ANGPTL4 concentrations with cancer risk did not differ by colorectal cancer subsite (HR colon cancer =

0.88, 95% CI = 0.81 to 0.96; HR rectal cancer = 0.97, 95% CI = 0.86 to 1.10; $P_{\text{het}} = 0.20$) and were consistent in lag analyses excluding participants within the first 2 and 5 years of follow-up (Figure 2A).

Prospective analyses in the UK Biobank included 860 incident colorectal cancer cases and 50 177 controls (median 14.2 year follow-up) (Table 3). Compared with those in the lowest quartile, participants in the highest quartile of baseline circulating ANGPTL4 concentrations had a higher mean BMI, lower levels of educational attainment, and spent less time in vigorous physical activity. In a model minimally adjusted for age and sex, we found evidence of a protective association of lower circulating ANGPTL4 concentrations with colorectal cancer risk (HR = 0.93, 95% CI = 0.86 to 0.99, $P = .03$). Findings did not materially change upon further adjustment for lifestyle (HR = 0.93, 95% CI = 0.87 to 1.00, $P = .06$) and dietary factors (HR = 0.93, 95% CI = 0.87 to 1.00, $P = .06$). Findings were similar across colon cancer (HR = 0.93, 95% CI = 0.86 to 1.01) and rectal cancer risk (HR = 0.92, 95% CI = 0.83 to 1.03; $P_{\text{het}} = 0.87$) and in “2 year” and “5 year” lag analyses (Figure 2B).

Impact of ANGPTL4 loss-of-function on colon differential gene expression and gene set enrichment

In gene-level analysis, we did not find evidence for an association of the loss-of-function p. E40K variant with differential gene expression after correcting for multiple testing (FDR $P < .05$) (Table S9). However, when exploring pathway-level enrichment using gene set enrichment analysis, differentially expressed genes ($P < .05$) were strongly enriched for 6 Hallmark gene sets (FDR $P < .05$). Down-regulated gene sets included those implicated in cellular proliferation (ie, targets of the E2F family of transcription factors, genes involved in the cell cycle G2/M checkpoint, and genes involved in mitotic spindle assembly), epithelial-mesenchymal transition (EMT), and bile acid metabolism (Figure 3, Table S10). There was one up-regulated gene set implicated in cellular proliferation (ie, genes regulated by the oncogenic MYC pathway).

Table 2. Characteristics of EPIC case-cohort study participants by quartiles of circulating ANGPTL4 concentrations (N = 5057).

Characteristic	ANGPTL4 concentrations			
	Q1 (N = 1265)	Q2 (N = 1264)	Q3 (N = 1264)	Q4 (N = 1264)
Age at recruitment, y	52.1 (9.1)	52.8 (8.6)	52.7 (8.7)	52.4 (8.7)
Female (%)	875 (69.2)	790 (62.5)	742 (58.7)	639 (50.6)
Body mass index, kg/m ²	26.7 (3.9)	27 (4.3)	26.9 (4.3)	27.2 (4.8)
Alcohol, g/day	10.8 (16.3)	13.1 (20.1)	13.9 (20.4)	16.5 (23.1)
Smoking (%)				
Never	687 (54.3)	626 (49.5)	600 (47.4)	604 (47.8)
Former	312 (24.7)	321 (25.4)	341 (27.0)	319 (25.2)
Current	266 (21.0)	317 (25.1)	324 (25.6)	341 (27.0)
Physical activity (%)				
Inactive	406 (32.1)	370 (29.2)	368 (29.1)	329 (26.0)
Moderately inactive	402 (31.8)	425 (33.6)	397 (31.4)	433 (34.3)
Moderately active	220 (17.4)	237 (18.8)	250 (19.8)	257 (20.3)
Active	219 (17.3)	217 (17.2)	233 (18.4)	243 (19.2)
Missing	18 (1.4)	15 (1.2)	16 (1.3)	3 (0.2)
Education level (%)				
None	236 (18.6)	199 (15.8)	191 (15.1)	174 (13.8)
Primary	480 (37.9)	492 (38.9)	452 (35.7)	456 (36.1)
Secondary	196 (15.5)	181 (14.3)	192 (15.2)	172 (13.6)
Technical/professional	169 (13.4)	212 (16.8)	197 (15.6)	219 (17.3)
Longer education	173 (13.7)	161 (12.7)	192 (15.2)	187 (14.8)
Not specified	11 (0.9)	19 (1.5)	40 (3.2)	55 (4.4)

Values are means and standard deviations for continuous variables and frequencies and percentages for categorical variables.

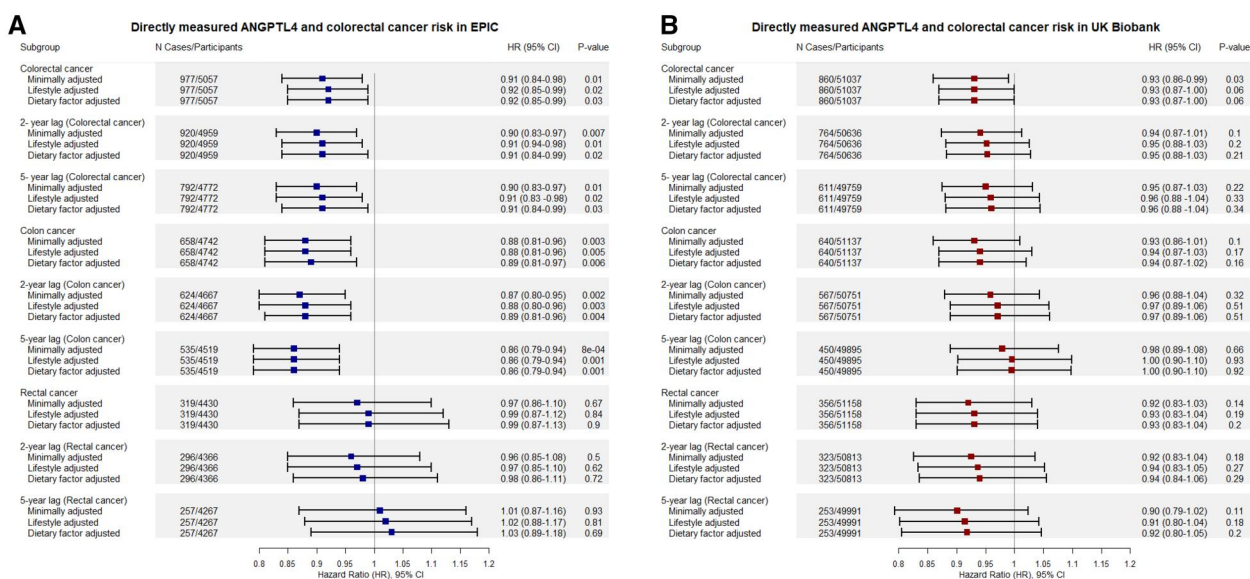


Figure 2. A) Minimally adjusted model was stratified on sex, center of origin, and age at recruitment. The lifestyle adjusted model was further adjusted for body mass index (BMI), alcohol (grams/day), smoking status (current, former, never smoker, unknown), physical activity index (inactive, moderately inactive, moderately active, active, missing), and highest level of education (not specified, none, primary, secondary, technical/professional, longer education). The dietary factor adjusted model was further adjusted for total daily energy intake, total daily red meat intake, total daily processed meat intake, total daily fiber intake, and total daily calcium intake. **B)** Minimally adjusted model was adjusted for sex and age at recruitment. The lifestyle adjusted model was further adjusted for BMI, alcohol consumption, smoking, physical activity, and education levels. The dietary factor adjusted model was further adjusted for red meat intake, processed meat intake, total daily energy intake, total daily calcium intake, and total daily fiber intake models were employed. 2-year lag = removed participants within the first 2 years of follow-up, 5-year lag = removed participants within the first 5 years of follow-up.

Table 3. Characteristics of UK Biobank prospective cohort study participants by quartiles of circulating ANGPTL4 concentrations (N = 51 291).

Characteristic	ANGPTL4 concentrations			
	Q1 (N = 12 825)	Q2 (N = 12 823)	Q3 (N = 12 821)	Q4 (N = 12 822)
Age at recruitment, y	54.7 (8.2)	56.4 (8.2)	57.4 (8.0)	58.6 (8.0)
Female (%)	7423 (57.9)	6886 (53.7)	6832 (53.3)	6532 (50.9)
Body mass index, kg/m ²	26.1 (4.0)	27.1 (4.3)	27.8 (4.7)	28.9 (5.6)
Alcohol (%)				
Never	1029 (8.0)	997 (7.8)	1094 (8.5)	1305 (10.2)
Special occasions only	1353 (10.5)	1356 (10.6)	1497 (11.7)	1805 (14.1)
1 to 3 times a month	1336 (10.4)	1324 (10.3)	1410 (11.0)	1501 (11.7)
Once or twice a week	3175 (24.8)	3395 (26.5)	3451 (26.9)	3277 (25.6)
3 or 4 times a week	3143 (24.5)	3016 (23.5)	2865 (22.3)	2526 (19.7)
Daily or almost daily	2762 (21.5)	2704 (21.1)	2472 (19.3)	2380 (18.6)
Missing or unknown	27 (0.2)	31 (0.2)	32 (0.2)	28 (0.2)
Smoking (%)				
Never	7328 (57.1)	7097 (55.3)	6835 (53.3)	6500 (50.7)
Previous	4142 (32.3)	4412 (34.4)	4513 (35.2)	4801 (37.4)
Current	1307 (10.2)	1249 (9.7)	1412 (11.0)	1446 (11.3)
Unknown or missing	48 (0.4)	65 (0.5)	61 (0.4)	75 (0.6)
Physical activity (min)				
Walking	1039.0 (1094.2)	1039.6 (1080.2)	1040.7 (1085.7)	1012.9 (1078.2)
Moderate	925.0 (1211.0)	909.7 (1199.1)	952.2 (1244.2)	920.8 (1224.3)
Vigorous	712.3 (1222.0)	648.5 (1130.2)	661.4 (1227.2)	627.9 (1182.8)
Education level (%)				
None	1638 (12.8)	1957 (15.3)	2349 (18.3)	3106 (24.2)
CSEs or equivalent	705 (5.5)	673 (5.2)	704 (5.5)	666 (5.2)
O levels/GCSEs or equivalent	2556 (19.9)	2645 (20.6)	2798 (21.8)	2655 (20.7)
NVQ or HND or HNC or equivalent	780 (6.1)	809 (6.3)	856 (6.7)	952 (7.4)
A levels/AS levels or equivalent	1515 (11.8)	1503 (11.7)	1361 (10.6)	1261 (9.8)
College or University degree	4851 (37.8)	4409 (34.4)	3918 (30.6)	3250 (25.3)
Other professional qualifications	630 (4.9)	671 (5.2)	683 (5.3)	717 (5.6)
Unknown or missing	150 (1.2)	156 (1.2)	152 (1.2)	215 (1.7)

Values are means and standard deviations for continuous variables and frequencies and percentages for categorical variables. Abbreviations: CSE = Certificate of Secondary Education, GCSE = General Certificates of Secondary Education, NVQ = National Vocational Qualification, HND = Higher National Diploma, HNC = Higher National Certificate, A levels = Advanced Level Qualification, AS = Advanced Subsidiary Level Qualification.

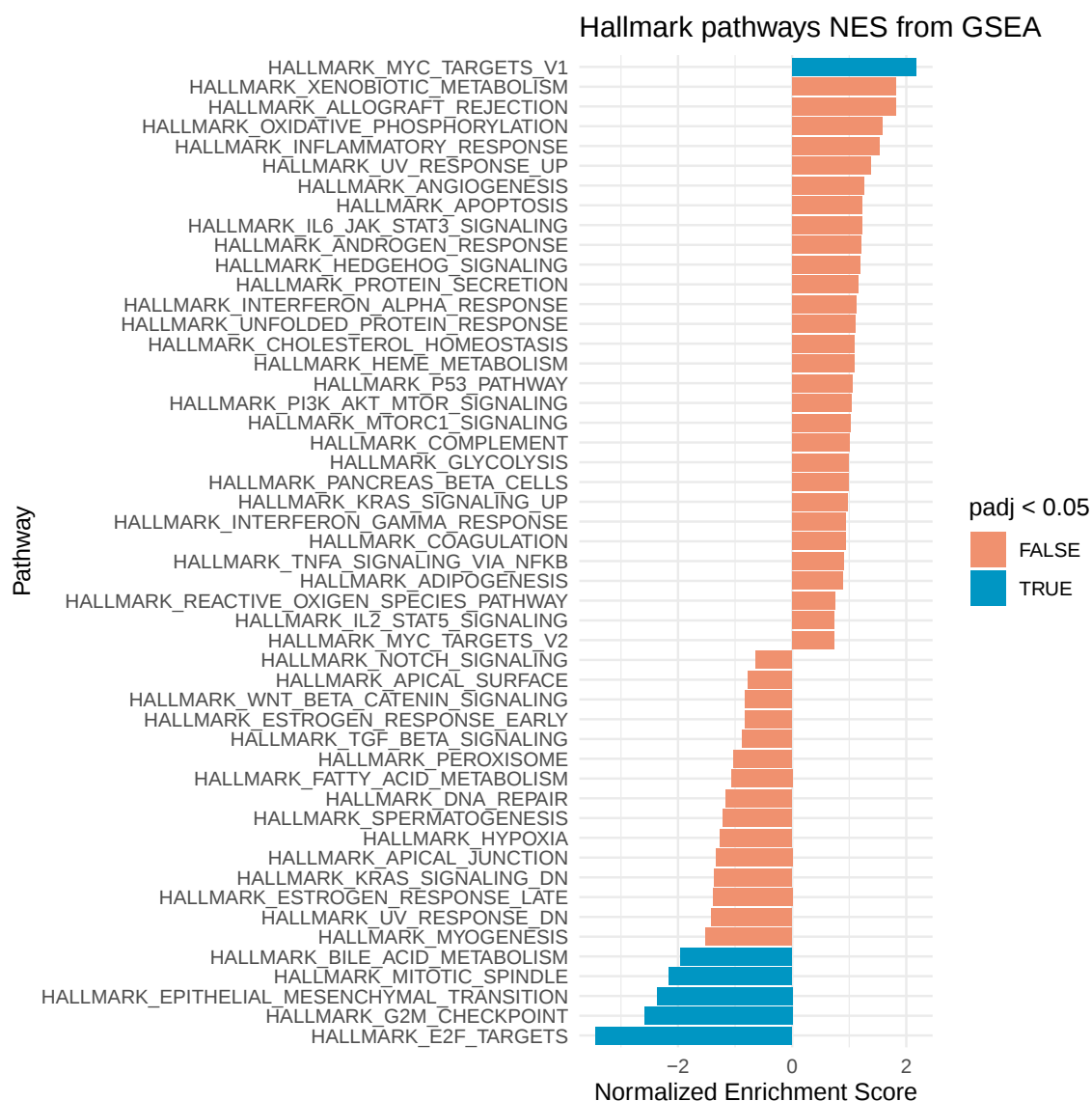


Figure 3. GSEA = Gene-set enrichment analysis.

Colon tumor ANGPTL4 expression and all-cause mortality

After a median follow-up of 1.8 (IQR 1.0-3.0) years of 465 colon cancer patients, 98 deaths were recorded, including 41 colorectal cancer deaths. In multivariable-adjusted Cox proportional hazards models, lower colon tumor ANGPTL4 gene expression was associated with reduced risk of all-cause mortality (HR per log2 decrease = 0.85, 95% CI = 0.73 to 0.99; $P = .04$). We also found that lower colon tumor ANGPTL4 expression was associated with reduced risk of colorectal cancer-specific mortality (HR = 0.66, 95% CI = 0.50 to 0.87; $P = 2.92 \times 10^{-3}$).

Discussion

Through triangulation of evidence across proteogenomic, observational, and molecular epidemiological analyses, we prioritize ANGPTL4 as a potential therapeutic target for colorectal cancer prevention. In combined drug-target Mendelian randomization and colocalization analyses of 78 473 cases and 107 143 controls, genetically proxied ANGPTL4 inhibition was associated with a reduced risk of colorectal cancer. In replication analyses using

independent prospective analysis of 977 incident colorectal cancer cases and 4080 non-cases in EPIC and 860 incident colorectal cancer cases and 50 177 controls in the UK Biobank, directly measured lower circulating ANGPTL4 concentrations were associated with reduced colorectal cancer risk. In gene set enrichment analysis of differential gene expression in 445 normal colon tissue samples, ANGPTL4 loss-of-function was associated with down-regulation of several cancer-related gene-sets, providing mechanistic insight into anti-tumorigenic properties of ANGPTL4. Finally, in analysis of 465 colon cancer patients, lower ANGPTL4 expression in colon tumor tissue was associated with a reduced risk of all-cause mortality. Collectively, these findings provide strong and consistent support for a potential role of ANGPTL4 in colorectal tumorigenesis. We found limited evidence to support differences in the association of ANGPTL4 with colon and rectal cancer risk in genetic studies though these analyses were likely underpowered to detect heterogeneity. In contrast, in analyses examining the association of directly measured ANGPTL4 across both EPIC and UK Biobank we found no evidence of heterogeneity of association, suggesting that limited evidence of heterogeneity in genetic studies could reflect a chance finding.

ANGPTL4 is a ubiquitously expressed glycoprotein that inhibits lipoprotein lipase and modulates fatty acid uptake in adipose and oxidative tissue.⁵⁶⁻⁶⁰ As a key regulator of triglyceride clearance, ANGPTL4 has emerged as an attractive therapeutic target for reducing triglyceride levels and adverse cardiovascular events.^{61,62} This is supported by human genetic evidence that loss-of-function variants in *ANGPTL4* are associated with lower plasma triglycerides and reduced coronary artery disease risk.⁶³ In addition, human genetic inactivation of *ANGPTL4* has been shown to improve glucose homeostasis and reduce type 2 diabetes risk.⁶⁴ At least 2 pharmacological *ANGPTL4* inhibitors are currently under Phase II clinical trial evaluation for their efficacy in lowering plasma triglycerides and reducing cardiovascular events.^{65,66}

Our findings potentially implicating *ANGPTL4* in colorectal cancer development recapitulate insights from preclinical studies. For example, *ANGPTL4* knockdown has been shown to inhibit proliferation, promote apoptosis, and suppress migration in colorectal cancer cell lines and to reduce colorectal tumor size in xenograft mouse models.^{67,68} Recombinant *ANGPTL4* has been reported to promote colon cancer growth by impairing CD8+ T cell activity in mice.⁶⁹ Recently, *ANGPTL4* suppression has been shown to reprogram endothelial cell metabolism and inhibit angiogenesis, providing another mechanism through which *ANGPTL4* may influence carcinogenesis.⁷⁰ Interestingly, prostaglandin E2, a putative key mediator of the effect of COX-2 on colorectal cancer, has also been reported to promote colorectal carcinoma cell proliferation via *ANGPTL4* under hypoxic conditions.^{71,72}

Consistent with prior reports, we did not find evidence of an association of genetically proxied triglyceride concentrations with colorectal cancer risk, suggesting that the association between *ANGPTL4* and colorectal cancer risk is mediated via pathways independent of triglyceride lowering.^{26,73} In gene set enrichment analysis, *ANGPTL4* loss-of-function lead to down-regulation of several biological pathways implicated in colon carcinogenesis including cellular proliferation, bile acid metabolism, and the EMT. For example, bile acids have been shown to promote colon cancer by damaging colonic epithelial cells, and inducing reactive oxygen species production, genomic destabilization, and apoptosis resistance.⁷⁴ In addition, the EMT has been reported to play an important role in colorectal cancer progression, metastasis, and drug resistance, and preclinical studies have suggested the efficacy of pharmacological perturbation of markers of the EMT in colorectal cancer.⁷⁵ Our findings thus validate and extend insights from preclinical models and can help to guide future work investigating mechanisms underpinning the effect of *ANGPTL4* on colorectal cancer development.

Contrary to some prior studies, we found little evidence to support associations of other lipid-perturbing targets (eg, PCSK9, CETP) with cancer risk (eg, breast, prostate, head and neck).^{27,28,76} The absence of or inconsistent application of colocalization analysis in some prior studies complicates assessment of whether discordance between findings reflects the presence of confounding by LD in previous studies, differences in instrument construction strategy across studies, or chance. Nonetheless, our findings suggesting little evidence of association of genetically proxied *ANGPTL3*, *APOC3*, *CETP*, and *PCSK9* inhibition with cancer risk may help to deprioritize further evaluation of these proteins as intervention targets for cancer prevention.

Strengths of this study include use of a triangulation framework leveraging genetic and conventional epidemiological approaches to strengthen causal inference. Notably, the consistency of findings across drug-target Mendelian randomization and conventional epidemiological analysis using independent

cohort studies, both of which may be susceptible to unrelated sources of bias, permitted us to increase confidence in our conclusions relating circulating *ANGPTL4* to colorectal cancer risk.⁷⁷ By leveraging gene expression data from normal and cancerous colon tissue samples we gained potential mechanistic insight into the effect of *ANGPTL4* on early precancerous changes in the colon and extended exploration of the role of *ANGPTL4* to mortality among colon cancer patients, supporting a possible role of this target across the carcinogenesis spectrum.

There are several limitations to this analysis. First, the validity of findings from cis-MR and gene expression analyses is dependent upon both exchangeability (ie, no confounding of the instrument-outcome association) and exclusion restriction (ie, no direct effect of the instrument on the outcome). Although various sensitivity analyses were performed to evaluate the robustness of findings to violations of both assumptions, these are unverifiable. Nonetheless, the use of the E40K predicted loss-of-function variant, that has been shown to directly influence *ANGPTL4* protein function, to proxy *ANGPTL4* inhibition along with evidence that circulating *ANGPTL4* and colorectal cancer risk share a causal variant within the *ANGPTL4* locus should minimize the likelihood that these assumptions have been violated.⁵¹ Second, conventional observational analyses performed in EPIC, the UK Biobank, and TCGA assume the absence of confounding, measurement error, and reverse causation though lag analyses in EPIC were consistent with the primary analysis. Third, genetic and conventional observational analyses are restricted to examining on-target (ie, target-mediated) effects of medications. Fourth, statistical power was likely limited in drug-target MR analyses of less common cancer subtypes. Fifth, genetic and conventional observational analyses were primarily performed in participants of European ancestry and, therefore, the generalizability of these findings to non-European populations is unclear. Sixth, we were unable to explore the association of both *ANGPTL4* loss-of-function with differential gene expression in normal rectal tissue because of the absence of suitable data in this tissue and *ANGPTL4* expression in rectal tumor samples with all-cause mortality due to the limited number of events in this dataset.

Colorectal cancer is the third most common cancer globally, accounting for more than 900 000 deaths in 2022.^{78,79} Aspirin and nonsteroidal anti-inflammatory drugs can be used to lower colorectal cancer risk in high-risk populations (eg, individuals with Lynch syndrome, familial adenomatous polyposis) but the increased risk of gastrointestinal bleeding on these medications limit their wider use.⁸⁰ There is therefore a need for identification of novel safe and effective chemoprevention agents for colorectal cancer to reduce the burden from this disease. Our findings leveraging genetic, observational, and molecular epidemiological designs recapitulate insights from preclinical studies indicating a protective effect of *ANGPTL4* inhibition in colorectal cancer risk. Further work validating findings in human studies and clarifying potential mechanisms of effect will guide further assessment of the viability of *ANGPTL4* inhibition as a therapeutic strategy for cancer prevention. In addition, investigation of the role of *ANGPTL4* in colorectal carcinogenesis in non-European populations will permit evaluation of the generalizability of these findings to other ancestries. Finally, ongoing clinical trials investigating pharmacological *ANGPTL4* inhibition for CVD present another opportunity to explore potential cancer preventive properties of these medications.

Conclusion

In conclusion, our comprehensive proteogenomic and observational analyses suggest a protective role of lowering circulating ANGPTL4 concentrations in colorectal cancer risk. These findings provide human validation to insights from preclinical studies and support the further evaluation of ANGPTL4 as a potential therapeutic target for colorectal cancer prevention.

Acknowledgments

The authors thank the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands, for their contribution and ongoing support to the EPIC Study. The authors also thank CERCA Programme, Generalitat de Catalunya for institutional support. The funders of this work played no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Author contributions

James Yarmolinsky (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing), Matthew Lee (Formal analysis, Writing—original draft, Writing—review & editing), Evelyn Lau (Formal analysis, Writing—review & editing), Ferran Moratalla-Navarro (Formal analysis, Writing—original draft, Writing—review & editing), Emma Vincent (Writing—review & editing), Ruifang Li-Gao (Data curation, Writing—review & editing), Patrick C.N. Rensen (Data curation, Writing—review & editing), Ko Willems van Dijk (Data curation, Writing—review & editing), Kostas Tsilidis (Writing—review & editing), Apiwat Sangphukieo (Data curation, Writing—review & editing), Elmira Ebrahimi (Data curation, Writing—review & editing), Jochen Hampe (Writing—review & editing), Loïc Le Marchand (Writing—review & editing), Franzel J.B. van Duijnhoven (Writing—review & editing), Kala Visvanathan (Writing—review & editing), Michael O. Woods (Writing—review & editing), Marcela Guevara (Writing—review & editing), Sabina Sieri (Writing—review & editing), Giovanna Masala (Writing—review & editing), Keren Papier (Writing—review & editing), Shama Virani (Data curation, Writing—review & editing), Tom Dudding (Data curation, Writing—review & editing), Abbas Dehghan (Writing—review & editing), Alexander G. Smith (Data curation, Formal analysis, Writing—review & editing), Dennis Wang (Writing—review & editing), Victor Moreno (Data curation, Writing—review & editing), Marc J. Gunter (Data curation, Writing—review & editing), and Ioanna Tzoulaki (Writing—review & editing).

Supplementary material

[Supplementary material](#) is available at *JNCI: Journal of the National Cancer Institute* online.

Funding

J.Y. and I.T. are supported by the National Institute for Health and Care Research Imperial Biomedical Research Centre. D.W. and E.L. are supported by A*STAR (UIBR), the Academy of Medical Sciences Professorship (APR7_1002), and the Engineering and Physical Sciences Research Council (EP/V029045/1). F.M.-N. and V.M. are supported by the Spanish Association Against Cancer (AECC)

Scientific Foundation grant GCTRA18022MORE, the Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), action Genrisk and the Instituto de Salud Carlos III (ISCIII), “Programa FORTALECE del Ministerio de Ciencia e Innovación,” through the project number FORT23/00032.

Conflicts of interest

The authors declare no potential conflicts of interest.

Data availability

Data supporting the findings of this study are available within the paper and its [supplementary information](#) files. Summary genetic association data on breast, ovarian, and prostate cancer can be obtained from the GWAS Catalog (accession numbers: GCST004988, 28346442, 29892016). Summary genetic association data on head and neck cancer will be posted on the GWAS Catalog with publication of Ebrahimi et al. medRxiv, 2024 (<https://www.medrxiv.org/content/10.1101/2024.11.18.24317473v1>). Summary genetic association data on colorectal cancer were obtained by submitting a data usage proposal to The Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) (<https://research.fredhutch.org/peters/en/genetics-and-epidemiology-of-colorectal-cancer-consortium.html>). Data from the EPIC study can be obtained by submitting a scientific proposal form (PF1) to the data access committee (<https://epic.iarc.fr/>).

Ethics statement

Analyses performed within the European Prospective Investigation into Cancer (EPIC) were approved by the International Agency for Research on Cancer (IARC) ethics committee (ethics approval number: IEC 17-39-A1).

Disclaimer

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

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