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Article

Human blood metabolites and the risk of colorectal cancer: A Mendelian randomization study

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Abstract: Background: Metabolomics can offer vital information into a cancer's condition. Despite its potential, research on the metabolites linked to colorectal cancer (CRC) remains limited. From a cell molecular biomechanics perspective, understanding these metabolite associations can offer a deeper understanding of the disease's underlying mechanisms. We performed Mendelian randomisation (MR) analyses to investigate causal associations between 486 blood metabolites and CRC. **Methods:** Data on blood metabolites were derived from a Genome-wide association study (GWAS) involving 7824 Europeans. Additionally, summary statistics for CRC were sourced from the FinnGen consortium database. To explore the causal relationship between CRC and blood metabolites, we primarily utilized the inverse variance weighted (IVW) analysis. Supplementary analyses incorporated MR-Egger and weighted median methods to ensure the robustness of our findings. The potential for pleiotropic effects was evaluated using the Cochran's Q test and the MR-Egger intercept test. Furthermore, colocalization analyses were performed to ascertain whether the observed associations were influenced by specific genetic loci within the genomic region. **Results:** The results of this study indicated significant associations between eight metabolites: Indolelactate (OR = 2.62, 95% confidence interval (CI): 0.26–1.66, p = 0.007), 1-heptadecanoylglycerophosphocholine (OR = 1.37, 95% CI: 0.10–0.54, p = 0.005), 1-stearoylglycerophosphocholine (OR = 3.47, 95% CI: 0.65–1.84, p = 0.00005) , X-11792 (OR = 0.57, 95% CI: −0.94–−0.17, p = 0.005), X-12038 (OR = 0.44, 95% CI: −1.50–−0. 12, p = 0.021), X-12212 (OR = 1.96, 95% CI: 0.10–1.25, p = 0. 022), X-14056 (OR = 0.50, 95% CI: −1.28–−0.12, p = 0.018) , X-14745 (OR 0.41, 95% CI: −1.48–−0.31, p = 0.003) and CRC. These metabolites might play roles in altering the mechanical properties of cells in the colon. They could potentially affect the cytoskeletal structure, cell membrane fluidity, or the way cells interact with the extracellular matrix. **Conclusion:** The eight identified blood metabolites with causative influence on CRC provide valuable clues for understanding CRC from a cell molecular biomechanics angle, which can further aid in its screening, prevention, and treatment strategies.

Keywords: blood metabolites; cell molecular biomechanics; colocalization analysis; colorectal cancer; Mendelian randomization

1. Introduction

Previous research indicates that cancer constitutes 21% of all deaths worldwide, placing colorectal cancer (CRC) as the second leading cause of mortality due to cancer [1]. Early-stage CRC patients have a five-year survival rate of about 90%,

which significantly diminishes to under 10% in cases of patients presenting with distant metastasis [2]. Therefore, enhancing early screening for CRC is crucial in preventing the progression of cancer. Despite studies exploring the metabolic pathways involved in colorectal carcinogenesis and progression [3], there is a lack of research on metabolic alterations in CRC. Investigating the connection between CRC and blood metabolites associated with CRC will be beneficial for the early detection, prevention, and treatment of CRC.

Metabolomics is a comprehensive examination of small molecule metabolites that can offer vital information into a cancer's condition. Specifically, changes in metabolite levels can be utilized to assist determine which modifications at the DNA, RNA, and protein levels lead to functional changes in cellular activity since they represent the activity of metabolic enzymes in cancer cells [4]. There is growing evidence that metabolic reprogramming, a key feature of cancer, is the outcome of numerous and frequently intricate interactions between signaling and metabolic pathways [4,5]. In addition, a growing area of research is that the selection of targeted medicines to meet the metabolic dependence of the cancer can be made more sensible with the use of metabolomics. This is exemplified by the fact that the oncometabolite D-2-hydroxyglutarate modifies the activity of chromatin-modifying enzymes. This was demonstrated to be significantly elevated in cells expressing isocitrate dehydrogenase mutations associated with cancer, and it was further demonstrated to be elevated in cells, tissues, and plasma from cancers containing somatic isocitrate dehydrogenase mutations [6–8]. Thus, metabolomics offers valuable insights for cancer screening, diagnosis, and treatment. However, little research has been conducted on CRC and metabolites linked to CRC.

Existing studies indicate that there is a positive correlation between metabolic syndrome, characterized by elevated levels of glucose in the bloodstream, and an increased likelihood of developing CRC [9]. Leucine, serotonin, imidazole propionate, and 2-linoleoylglycerol (18:2) are metabolites associated with CRC [10]. However, no overall association between serum metabolites and CRC was observed in the study by Amanda et al. [11]. The above phenomenon may be caused by factors, for instance, changes in metabolite content may occur in cancer patients after treatment, or patients may have other pre-existing diseases and have been taking medication for a long time before being diagnosed with cancer. Additionally, the presence of a tumor can also cause changes in metabolite content. These factors can result in an inaccurate relationship between CRC and metabolites, necessitating further research and evidence.

MR is a statistical technique that use SNPs as IVs to establish causal relationships between modifiable exposures and outcomes using non-experimental data. The alleles of a certain SNP are allocated to gametes during meiosis in a random manner. As a result, they are not often affected by reverse causality or residual confounding. This random assignment is comparable to how exposure is assigned in the population [12]. Consequently, MR has become an essential tool for scientists to explore causal links between risk variables and outcomes using observational data [13].

This study primarily use MR and colocalization analysis to examine the causal association between 486 blood metabolites and CRC. Ultimately, it identifies 8

blood metabolites that have a causative link with CRC, which has implications for the prevention and screening of CRC.

2. Materials and methods

2.1. Study design and date source

For a robust MR study, it is essential to meet three fundamental assumptions: (1) The instrumental variables must exhibit a solid link to the exposures under investigation; (2) These variables must be impervious to the effects of confounding elements; (3) The instrumental variables should exert their influence on outcomes exclusively through the exposures, excluding any immediate connection. In the present study, the MR analyses were methodically executed with R software (version 4.2.1), leveraging the TwoSampleMR and coloc packages to ensure the integrity of our findings.

In the current research, we leveraged the blood metabolite data provided by Shin and colleagues [14]. Access to comprehensive summary statistics was granted through the Metabolomics GWAS Server (https://metabolomics.helmholtz-muenchen.de/gwas/). Our cohort encompassed a total of 7824 European participants, with 1768 individuals sourced from the KORA F4 study and an additional 6056 from the UK Twin Study. Post-quality assurance procedures, we conducted an analysis encompassing 486 metabolites, which included both 309 identified and 177 yet-to-be-identified metabolites, as referenced in Supplementary materials **Table S1**. The unidentified metabolites are labeled with an "X" to signify their obscure chemical attributes. The screening basis of 486 metabolites is as follows: (1) Involvement in specific metabolic pathways. Tryptophan metabolic pathway. Tryptophan metabolites such as indole lactic acid and indole-3-lactic acid have been widely studied and are related to inflammation and oxidative stress in CRC. Phospholipid metabolic pathway. Lysophosphatidylcholine (LPC) and its isomers, such as LPC (17:0) and LPC (18:0), are involved in cell membrane fluidity and signal transduction and are closely related to the risk of CRC. Amino acid metabolism. Amino acids such as leucine and glutamine play an important role in tumor metabolism and affect cell proliferation and energy metabolism [3]. Lipid metabolism. Lipid metabolites such as linoleic acid and eicosapentaenoic acid (EPA) are related to inflammation and oxidative stress and affect the development of CRC [10]; (2) Related to tumor metabolic reprogramming. Glycolysis. Tumor cells often obtain energy through the glycolysis pathway, even in the presence of sufficient oxygen. Related metabolites such as lactate and pyruvate may be related to the risk of CRC [8]. Fatty acid metabolism: Tumor cells require a large amount of fatty acids to support cell membrane synthesis and energy metabolism. Related metabolites such as palmitic acid and stearic acid may affect the risk of CRC [9]. Amino acid metabolism: Tumor cells have an increased demand for certain amino acids, such as glutamine and leucine. Changes in the levels of these metabolites may reflect the metabolic reprogramming of CRC [10]; (3) Literature support: According to previous studies, some metabolites have been reported to be associated with the risk of CRC. For example, metabolites such as leucine, serotonin, imidazole propionate, and 2-linoleoylglycerol (18:2) are associated with the occurrence and development of CRC [10]. Although the

specific functions of some unknown metabolites are unclear, they have been found to be associated with disease risk in other types of cancer, suggesting that they may also play an important role in CRC [11].

A set of summary-level statistics on CRC was obtained from the FinnGen consortium database (R9 release) [15], which included 6509 cases and 287,137 controls. The identification method of CRC cases in the FinnGen database is as follows: CRC cases are identified by International Classification of Diseases (ICD) codes, mainly using ICD-10 codes C18–C20 (colon cancer, rectal cancer, and colorectal cancer). The diagnosis of the case is confirmed by hospital records, pathology reports, and clinician's diagnosis. The FinnGen project works closely with the Finnish National Health Information System to ensure the accuracy and completeness of the data. The follow-up period of the study subjects was from 1 January 1987 to 31 December 2018, covering a follow-up period of up to 32 years. The follow-up data includes patients' medical records, pathology reports, and death registration information, ensuring comprehensive tracking of CRC cases.

2.2. IVs selection

To satisfy the three hypotheses of MR studies, we performed a rigorous screening of IVs related to blood metabolites from multiple perspectives. First, we identified several single nucleotide polymorphisms (SNPs) that were significantly associated ($p < 1 \times 10^{-5}$) with blood metabolites; secondly, we prune by eliminating linkage disequilibrium (LD), setting a threshold of $R2 < 0.01$ and within 10,000 kb, to obtain independent SNPs. This step is to ensure the independence between the selected IVs and avoid false positive results due to LD; third, in order to ensure that the selected IVs have sufficient strength, we performed a test using the F statistic, and selected SNPs with an F statistic greater than 10 to mitigate possible bias due to the weak testing power of the tool [16]. The F statistic is an indicator of the effectiveness of instrumental variables. A low F statistic may mean that the instrumental variables are not strong enough, resulting in less accurate estimation results. Subsequently, we reconciled the exposure and outcome data to ensure that the IV effects on exposure and outcomes corresponded to the same allele. In addition, we removed SNPs that exhibited palindromic effects and allelic discordance. Finally, we extracted SNPs associated with blood metabolites from the obtained results and eliminated SNPs significantly associated with outcomes ($p < 1 \times 10^{-5}$) to avoid potential confounding factors affecting the results of MR analysis. Through this series of screening steps, we ensured the quality and suitability of the selected IVs, laying a solid foundation for subsequent MR analysis.

2.3. Statistical analysis and sensitivity analysis

In this research, we deployed the Inverse Variance Weighted (IVW), Weighted Median (WM), and MR-Egger approaches from the TwoSampleMR suite to appraise the causal relationship between blood metabolites and the development of Colorectal Cancer (CRC), with a focus on counteracting the pleiotropic influences of genetic variation. At the outset, the IVW technique was engaged as our principal analytical instrument, amalgamating the Wald ratio estimates of each SNP in relation to the

outcome to produce an aggregated causal estimate, thereby enhancing statistical strength [17]. The data obtained were instrumental in conducting a preliminary evaluation of the causal influence that blood metabolites exert on CRC. Supplementary analyses were executed using the WM and MR-Egger methodologies to probe for biases that might arise from substandard IVs and horizontal pleiotropy effects [17,18].

To substantiate the dependability of our conclusions, we initiated sensitivity analyses through two distinct analytical strategies: The Cochran's Q test and the MR-Egger intercept examination [19]. The Cochran's Q test is instrumental in evaluating the variability in the magnitude of effects among the instruments within correlated samples, thereby informing the decision between a fixed or random-effects IVW approach. A significant Cochran's Q test result, indicated by a *p* value below 0.05, points to the presence of heterogeneity. Furthermore, the MR-Egger intercept examination is utilized to detect any directional pleiotropy and bias that could result from the use of invalid IVs.

2.4. Colocalization analysis

Utilizing the coloc R package, we performed colocalization analysis to delve deeper into the possibility that the interactions of identified metabolites with Colorectal Cancer (CRC) might be dictated by genetic loci within specific genomic zones [20]. Data from the eQTLGen Consortium, which includes comprehensive cis-expression quantitative trait loci (eQTL) for blood-based gene expression across 31,684 samples (https://www.eqtlgen.org/), was leveraged. The genetic predictions' correlation for both phenotypes can be interpreted through one of five plausible models: H0 signifies no relationship of the SNP within the locus to any phenotype; H1 and H2 suggest a singular relationship of the SNP to a single phenotype; H3 indicates a connection of the SNP to both phenotypes, albeit without a relationship between the phenotypes themselves; H4 denotes a shared SNP linking both phenotypes and the SNP in question. The colocalization analysis is particularly focused on evaluating the posterior probability of model H4.

3. Results

3.1. Causal association of blood metabolites with CRC

In this study, we conducted a comprehensive assessment of 486 serum metabolites in relation to CRC risk using a rigorous MR design. The filtered IVs contained between 3 and 502 SNPs. All SNPs linked to metabolites had F statistics more than 10, indicating the substantial power of IVs (Supplementary materials **Table S2**). Next, based on the results of the IVW analysis, 29 metabolites were identified, 16 of which have a known chemical identity and the other 13 of which have an unknown chemical identity, that may have causative impacts on CRC (Supplementary materials **Table S3**). The known metabolites are classified as amino acids, cofactors and vitamins, lipids, and xenobiotics.

Following a consolidation of supplementary and sensitivity evaluations, our analysis pinpointed nine blood metabolites that adhered to rigorous selection

parameters (as depicted in **Figure 1**). To be precise, augmented concentrations of indolelactate (OR = 2.62, 95% CI: 0.26−1.66, p = 0.007), 1-heptadecanoylglycerophosphocholine (OR = 1.37, 95% CI: 0.10−0.54, p = 0.005), 1-stearoylglycerophosphocholine (OR = 3.47, 95% CI: 0.65−1.84, p = 0.0005), and the metabolite X-12212 (OR = 1.96, 95% CI: 0.10−1.25, p = 0.022) were linked to an increased propensity for developing Colorectal Cancer (CRC). In contrast, increased levels of X-11792 (OR = 0.57, 95% CI: −0.94 to −0.17, p = 0.005), X-12038 (OR = 0.44, 95% CI: −1.50 to −0.12, p = 0.021), X-14056 (OR = 0.50, 95% CI: −1.28 to −0.12, p = 0.018), and X-14745 (OR = 0.41, 95% CI: −1.48 to −0.31, p = 0.003) were associated with a diminished risk of CRC (for details, refer to **Table 1**).

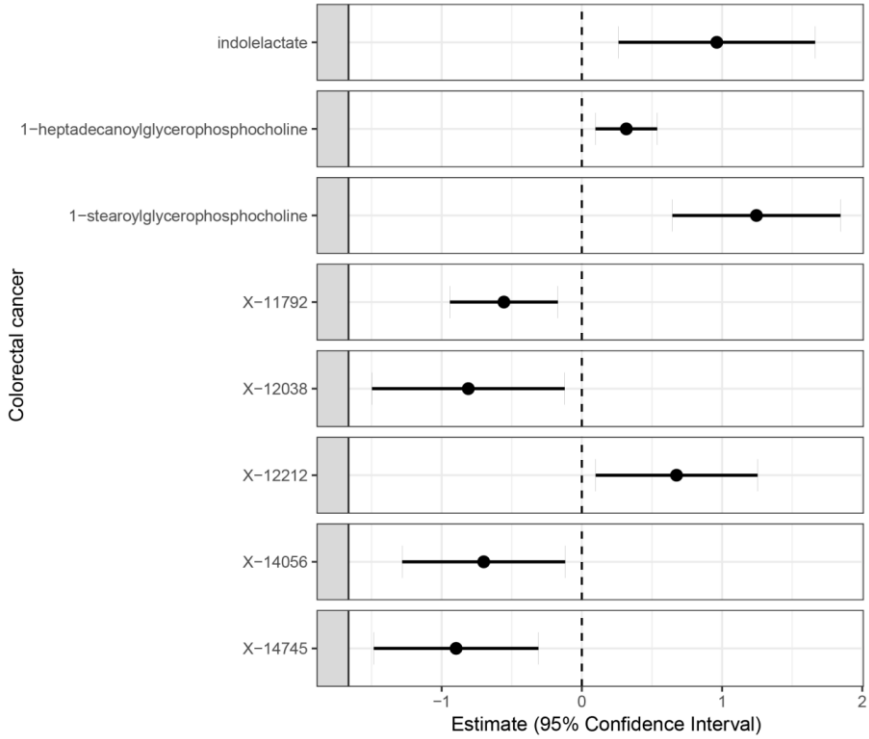

Figure 1. Forest plot for the causality of blood metabolites on colorectal cancer.

Table 1. MR analysis for causality from blood metabolites on colorectal cancer.

Metabolites	N	MR analysis			Heterogeneity		Pleiotropy
		Methods	OR (95% CI)	*p*	Q	*p*	*p*
indolelactate	21	IVW	2.62 (0.26–1.66)	0.007	17.70	0.41	0.92
		ME	2.25 (−2.40–4.02)	0.600	17.69	0.34	
		WM	5.18 (0.64–2.65)	0.001			
1-heptadecanoylglycerophosphocholine	13	IVW	1.37 (0.10–0.54)	0.005	9.64	0.56	0.60
		ME	1.81 (−0.58–1.77)	0.286	9.37	0.50	
		WM	1.49 (0.09–0.71)	0.011			
1-stearoylglycerophosphocholine	51	IVW	3.47 (0.65–1.84)	0.00005	23.12	0.10	0.70
		ME	6.87 (−1.57–5.43)	0.280	22.97	0.10	
		WM	2.99 (0.27–1.91)	0.009			

Table 1. (*Continued*).

Metabolites	N	MR analysis			Heterogeneity		Pleiotropy
		Methods	OR (95% CI)	*p*	Q	*p*	*p*
X-11792	10	IVW	0.57 (−0.94–−0.17)	0.005	5.63	0.78	0.60
		ME	0.76 (−1.42–0.88)	0.645	5.36	0.72	
		WM	0.56 (−1.09–−0.06)	0.030			
X-12038	10	IVW	0.44 (−1.50–−0.12)	0.021	3.06	0.93	0.84
		ME	0.55 (−2.85–1.67)	0.608	3.02	0.88	
		WM	0.37 (−1.89–−0.11)	0.028			
X-12212	14	IVW	1.96 (0.10–1.25)	0. 022	13.97	0.30	0.33
		ME	3.52 (−0.19–2.71)	0.083	12.76	0.31	
		WM	2.56 (0.06–1.82)	0.036			
X-14056	10	IVW	0.50 (−1.28–−0.12)	0.018	7.36	0.50	0.97
		ME	0.48 (−2.34–0.89)	0.323	7.35	0.39	
		WM	0.39 (−1.72–−0.16)	0.018			
X-14745	14	IVW	0.41 (−1.48–−0.31)	0.003	12.79	0.38	0.61
		ME	1.01 (−3.80–3.81)	0.997	12.47	0.33	
		WM	0.41 (−1.78–−0.10)	0.028			

Abbreviations: CI, confidence interval; IVW, inverse variance weighted; ME, MR-Egger; N, number of single nucleotide polymorphisms; OR, odds ratio; WM, weighted median.

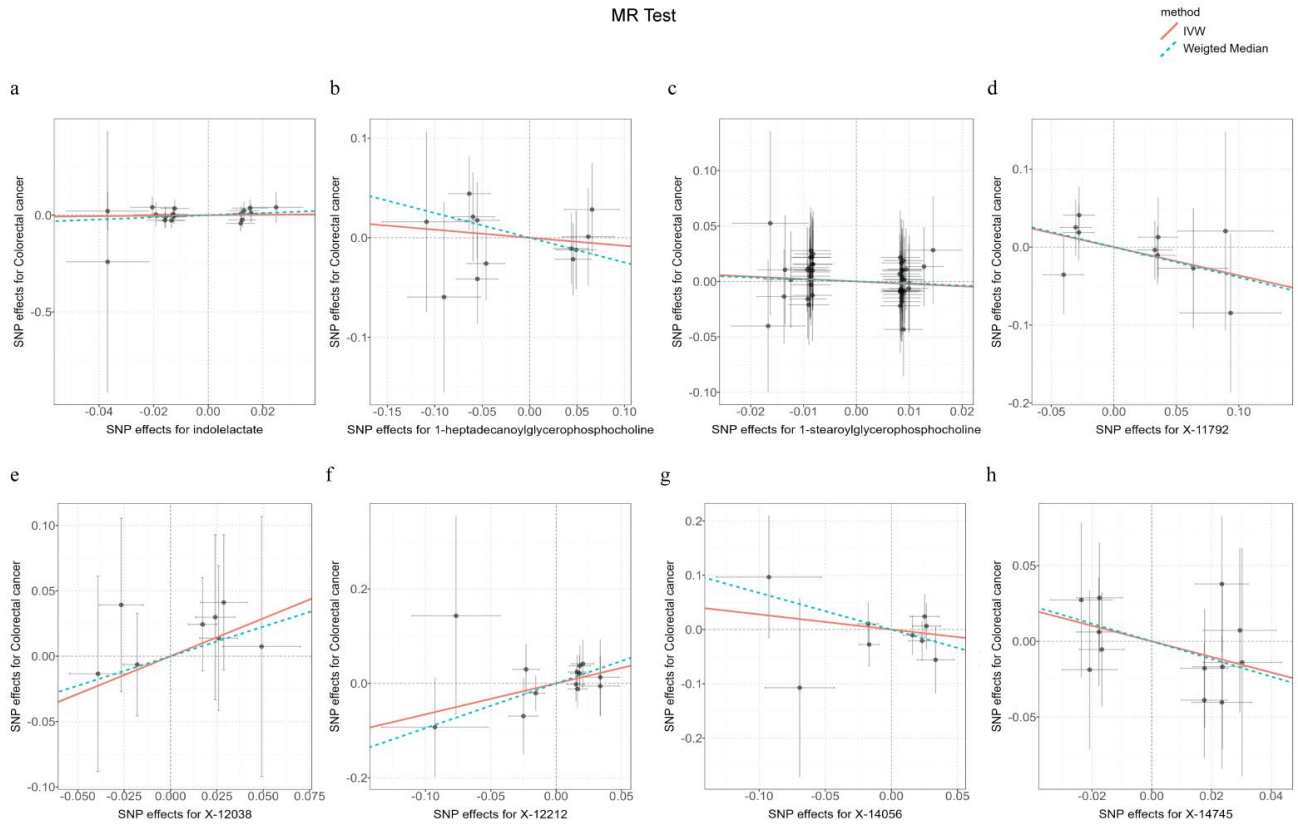

Figure 2. Scatterplot of significantly associated (IVW derived $p < 0.05$) and directionally consistent estimates. SNP, single nucleotide polymorphisms.

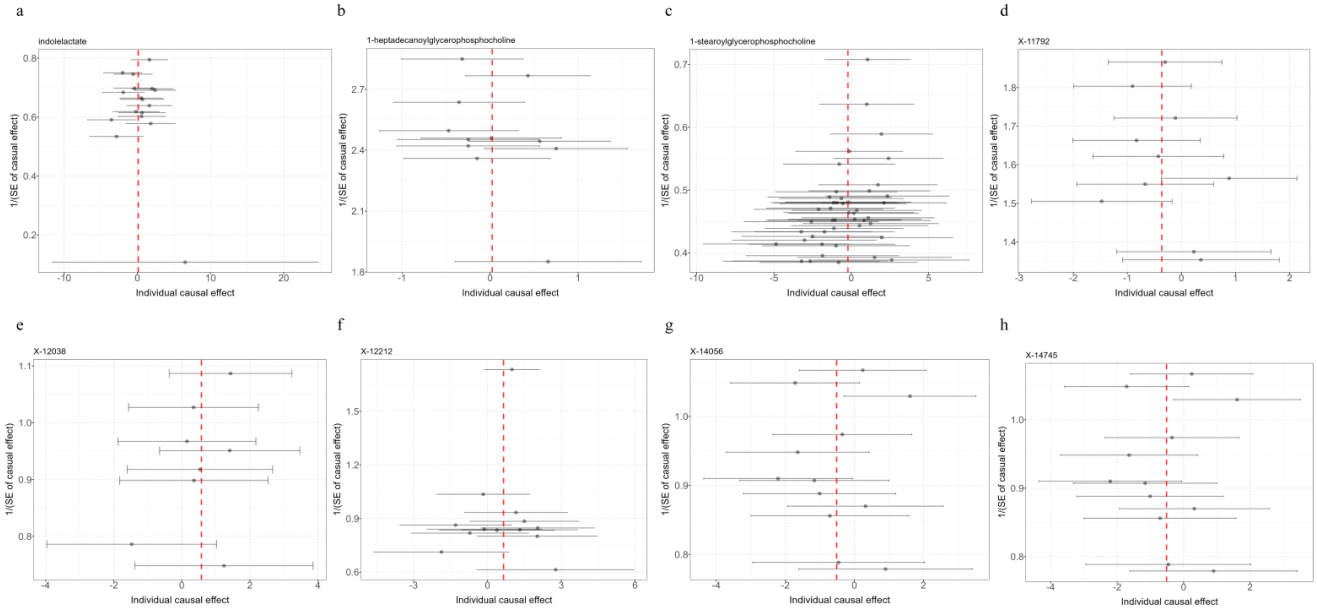

Figure 3. Funnel plot to assess heterogeneity. The red line represents the inverse-variance weighted estimate.

Conclusively, the IVW estimates demonstrate statistical significance ($p < 0.05$), and there is a concordance in the directionality and magnitude between IVW and WM estimates, thereby bolstering confidence in the stability of the causal nexus (as depicted in **Figure 2**). In contrast, the MR-Egger examination failed to establish a causal connection between blood metabolites and Colorectal Cancer (CRC) ($p > 0.05$; for details, see **Table 1**). The MR analysis outcomes potentially indicate a causal relationship between blood metabolites and CRC, given that the weighted median approach to estimation surpasses the MR-Egger analysis in terms of precision [21]. Significantly, neither the MR-Egger intercept test ($p > 0.05$) nor Cochran's Q test ($p > 0.05$) uncovered any signs of pleiotropic influences or variability in genetic effect sizes, which robustly underscores the reliability of our analytical approach (as detailed in **Table 1**). While the funnel plot's symmetry could imply a risk of horizontal pleiotropy that might skew MR methodologies, it is notable that the plot did not exhibit any asymmetry (as illustrated in **Figure 3**).

3.2. Colocalization analysis

After confirming the potential causal relationships between the above eight blood metabolites and CRC, we performed a co-localisation analysis to validate whether the genetic association between metabolites and CRC was due to shared causal genetic variants. The results of the co-localisation analysis showed no evidence of common localisation between the eight blood metabolites and the CRC association (PP4 < 80%), suggesting that the observed associations are unlikely to be due to confounding effects of common causal SNPs (Supplementary materials **Table S4**).

4. Discussion

For the present research, we amalgamated two extensive Genome-Wide

Association Study (GWAS) datasets to delve into the causal implications of 486 blood metabolites on Colorectal Cancer (CRC) through a Mendelian Randomization (MR) approach that focused on Inverse Variance Weighted (IVW), MR-Egger, and Weighted Median methodologies. Our findings revealed that indolelactate, as dictated by genetics, along with 1-heptadecanoylglycerophosphocholine (LPC (17:0)), 1-stearoylglycerophosphocholine (LPC (18:0)), and X-12212 are correlated with a higher propensity for CRC. In contrast, X-11792, X-12038, X-14056, and X-14745 are associated with a diminished risk for CRC.

The surge in both the incidence and fatality of Colorectal Cancer (CRC) in recent times has cast a heavy shadow over global well-being [22], underscoring an urgent call for the creation of accessible and powerful biomarkers. The visual representation of biological processes through blood metabolites is akin to a snapshot, encapsulating the interplay of internal and external factors [22]. A wealth of emerging research points to the involvement of blood metabolites in the biological underpinnings of CRC, though a concrete causal link remains to be firmly established. In light of this, our team embarked on a Mendelian Randomization (MR) study focusing on the Finnish demographic, with the ambition of decoding the causal ties between blood metabolites and CRC, and by extension, charting a course for future screening and treatment protocols for CRC.

Indolelactate, a tryptophan metabolite, is found in human plasma, serum, and urine [23]. Tryptophan is metabolized by indoles and kynurenine pathways. Biomarkers are usually measured in serum or urine but may be limited by their distance from the intestinal mucosa where CRC develops. Fecal metabolomics might be more effective due to their proximity to the colorectal mucosa and interaction with the microbiota [24]. Fecal [24] and mucosal [25] metabolomic analyses detected tryptophan and N-acetyltryptophan, linked to CRC risk. We hypothesized that tryptophan mediates indolelactate to promote CRC. In the context of CRC, abnormal activation of the tryptophan metabolic pathway may lead to an aggravated inflammatory response in the intestinal microenvironment, because tryptophan metabolites can directly or indirectly activate immune cells, leading to the release of proinflammatory cytokines, thereby promoting the formation of a chronic inflammatory state. This chronic inflammatory environment provides favorable conditions for the growth and invasion of tumor cells [23]. In addition, abnormal activation of the tryptophan metabolic pathway may also increase the level of oxidative stress. Oxidative stress refers to the imbalance between the excessive production of reactive oxygen species (ROS) in cells and the antioxidant defense mechanism [25]. In CRC, oxidative stress can lead to DNA damage, oxidative modification of proteins and lipids, and these changes may promote cell transformation and tumor progression. Indole lactic acid, as a tryptophan metabolite, may directly participate in the development of CRC by affecting the function of intestinal epithelial cells, including the integrity of the intestinal barrier and the regulation of cell proliferation [22]. Impairment of intestinal barrier function may lead to the translocation of bacteria and toxins, further aggravating the inflammatory response and oxidative stress, while uncontrolled cell proliferation may lead to tumor formation and growth [24,25]. Therefore, regulation of the tryptophan metabolic pathway may become a potential target for the prevention and treatment of CRC.

However, an animal study [26] found that indole-3-lactate (ILA), structurally similar to indolelactate, reduces colorectal tumorigenesis by enhancing CD8+ T cells' antitumor immunity [26]. Most of these studies were based on metabolite levels in serum or urine, which may be affected by confounding factors, such as patients' dietary habits, lifestyles, and medication use. Our MR analysis reduced the effects of reverse causality and confounding factors by using genetic instrumental variables, thus providing stronger evidence that indole lactic acid is associated with a higher risk of CRC. This finding is different from the results of previous observational studies, suggesting that the role of indole lactic acid in CRC may be more complex and requires further research to verify.

LPC is a metabolite of phosphatidylcholine and is involved in inflammation and cell signaling. LPC (17:0) and LPC (18:0) are isoforms of lysophosphatidylcholine (LPC) differing only at the C-1 position, with heptadecanoic acid and stearic acid chains, respectively. The distinct mechanisms and effects of these isoforms in colorectal cancer (CRC) are not well-studied. Studies have shown that LPC (17:0) and LPC (18:0) may affect cell proliferation and apoptosis by affecting cell membrane fluidity and signal transduction pathways, such as G protein-coupled receptors. These metabolites may also increase the risk of CRC by promoting inflammatory responses and oxidative stress [27]. LPC is a biologically active lipid metabolite of phosphatidylcholine, primarily produced by secretory phospholipase A2, HDL-associated lecithin-cholesterol acyltransferase, hepatic and endothelial lipases, and during lipoprotein oxidation [27]. The major types of LPC—LPC (16:0), LPC (18:1), LPC (20:4), and LPC (22:6)—account for 90% of total plasma LPC levels [28]. In cells, LPC concentrations are lower than their corresponding phospholipids due to active acyl chain doping by lysophosphoryltransferases [28]. In contrast, LPC is abundant in tissue fluid and plasma [28]. Plasma LPC is mainly secreted by the liver and exists largely in a less active albumin-bound form, with its secretion mechanisms still uncharacterized [28].

The physiological actions of LPC are mediated through S1P2 and S1P3 receptors coupled to Gi, Gq, and G12/13 proteins, activating PLCs and increasing intracellular Ca2+, which stimulates ERK, Rho, and Rac [29]. The LPA-GPCR, LPA1, LPA2, and LPA3 receptors also link to these signaling pathways, promoting cell survival via Akt [29]. LPC's effects vary with its biochemical structure: Saturated LPC (16:0) and LPC (18:0) and monounsaturated LPC (18:1) are pro-inflammatory, inducing monocyte chemotaxis and macrophage production of pro-inflammatory cytokines, while unsaturated LPC (22:4) and LPC (22:6) are anti-inflammatory [28]. LPC (18:0) is a CRC risk factor, but the effects of LPC could also stem from its metabolites [28].

Few studies address LPC's impact on intestinal tissue. Some findings suggest LPC damages ileum mucosa cells [30] and promotes pro-inflammatory cytokine release in colon tissue, damaging the epithelial barrier [31]. A lipidomic analysis of CRC patients showed significant differences in glycerophospholipids, particularly LPC, compared to normal tissues [32]. A CRC animal study indicated that high-fat diets (HFD) alter glycerophospholipid metabolism, upregulating LPC and lysophosphatidic acid (LPA) [33]. Elevated LPA promotes CRC by accelerating the cell cycle and compromising the intestinal barrier, suggesting LPC may contribute to

CRC through conversion to LPA [28]. Our MR analysis provided the first evidence of a causal relationship between these unknown metabolites and CRC risk. Although the specific mechanisms of these metabolites require further study, this finding provides a new direction for future metabolomics research. X-11792 and X-12038 may reduce CRC risk by regulating the cell cycle and inhibiting cell proliferation, while X-12212 may increase CRC risk by promoting cell proliferation and inhibiting apoptosis. X-14056 and X-14745 may reduce CRC risk by regulating immune responses and the production of inflammatory mediators.

Recent studies, however, link lower LPC plasma levels to unfavorable disease outcomes, showing that LPC substances (16:0, 18:0, and 18:1) inhibit reactive oxygen species production, neutrophil activation, and histamine release, acting as membrane stabilizers [27]. This contradicts earlier findings, highlighting LPC's complex roles in CRC, which may vary by subtype and tumor microenvironment.

LPC (17:0) has been less studied, with one study indicating it stimulates intestinal receptors that activate GLP-1, promoting insulin secretion and reducing metabolic disorders [34]. This suggests LPC (17:0) could be an important CRC biomarker, but further studies are needed.

Upstream, lecithin (PC) influences cancer cell signaling. LPCAT1 increases saturated PC in membranes, enhancing proliferative signals, while LPCAT3 reduces saturated PC to mitigate tumor outcomes [35]. Different LPCATs thus control membrane structure and signaling activity by modulating PC saturation, adding to LPC's complexity in cancer. Saturated PCs are linked to aggressive histology and poor outcomes in breast cancer patients [36], but their connection to CRC is under-researched. Given conflicting data on LPC's role in CRC, investigating its upstream and downstream pathways is essential for understanding its role in cancer.

For unknown metabolites such as X-11792, X-12038, X-12212, X-14056, and X-14745, the potential mechanism between them and CRC is difficult to determine due to the lack of specific chemical structure and biological function information. However, some hypotheses can be put forward that the two metabolites X-11792 and X-12038 may be associated with a reduced risk of CRC. They may be involved in regulating the cell cycle, inhibiting cell proliferation, or promoting cell apoptosis, thereby slowing down tumor development. X-12212 is associated with an increased risk of CRC and may act by promoting cell proliferation, inhibiting apoptosis, or affecting DNA repair mechanisms. The two metabolites X-14056 and X-14745 may reduce the risk of CRC by regulating immune responses, affecting the production of inflammatory mediators, or changing the composition of the intestinal microbiota. For unknown metabolites, further research is needed to determine their chemical structure and biological function. This may include metabolite identification using mass spectrometry, gene expression analysis to explore their potential roles in cells, and animal models and in vitro cell experiments to study their effects on tumor development. In addition, studying the expression patterns of these metabolites in CRC patients and healthy people, as well as their interactions with known CRC risk factors (such as dietary habits, genetic factors, and lifestyle) are also important directions for future research. Through these studies, we can better understand the role of these metabolites in the development of CRC and provide new targets for the prevention and treatment of CRC.

According to the above studies, many metabolites affect the risk of CRC by regulating inflammatory responses. For example, indole lactic acid and indole-3-lactic acid can enhance the anti-tumor activity of immune cells and reduce chronic inflammatory states, thereby reducing the risk of CRC [26]. Based on the metabolite levels in serum or urine, it was found that ILA can enhance the anti-tumor immunity of CD8+ T cells, thereby reducing the risk of CRC. I3LA has also been reported to be negatively correlated with CRC risk, which may play a role by regulating intestinal microbiota and reducing inflammatory responses [27]. Studies have found that LPC (17:0) levels are positively correlated with CRC risk, especially in people with a high-fat diet [33]. LPC (17:0) may increase the risk of CRC by affecting cell membrane fluidity and signal transduction pathways such as G protein-coupled receptors (GPCRs), promoting inflammatory responses and oxidative stress. LPC (18:0) is also positively correlated with CRC risk, and its mechanism may be similar to that of LPC (17:0) [34]. Leucine is one of the branched-chain amino acids and has been reported to be associated with an increased risk of CRC [35]. Leucine may increase the risk of CRC by activating the mTOR signaling pathway, promoting cell proliferation and inhibiting cell apoptosis. Glutamine is an important energy source for tumor cells, and its elevated level may be associated with the development of CRC [36]. Metabolites such as linoleic acid and EPA affect the development of CRC by affecting the level of oxidative stress in cells, thereby affecting DNA damage and cell apoptosis [37]. EPA is an omega-3 fatty acid that has been reported to be associated with a lower risk of CRC, possibly by reducing inflammatory responses and inhibiting cell proliferation [38]. In addition, metabolic reprogramming is an important feature of CRC, and many metabolites support tumor growth and survival by affecting cell energy metabolism and material metabolism.

Although previous observational studies and mechanistic studies have provided rich evidence, most of these studies are based on cross-sectional or prospective cohort designs, with potential confounding factors and reverse causality, and cannot provide strong causal evidence. This study used a Mendelian randomization (MR) design and genetic instrumental variables to reduce the impact of confounding factors and reverse causality, providing stronger evidence for the causal relationship between metabolites and CRC. The innovation of this study is that it is the first time to use MR design to systematically explore the causal relationship between 486 blood metabolites and CRC through genetic instrumental variables. This provides a new perspective for understanding the role of metabolites in the occurrence of CRC. In addition, two large-scale GWAS data, including blood metabolite data provided by Shin et al. and CRC data in the FinnGen Alliance database, were integrated to ensure the sample size and statistical power of the study. This study not only focuses on known metabolites, but also explores the potential role of unknown metabolites in CRC, providing new directions for future research.

This MR analysis offers several advantages. First, MR can assess the causal impact of risk factors on outcomes using genetic IVs, reducing confounding and reverse causation [37,38]. Second, this study is comprehensive, analyzing 486 blood metabolites for their causal relationship with CRC. Third, we used LDSC to assess heritability and genetic correlation, enhancing the robustness of MR estimates. We also conducted co-localization analyses to link GWAS signals to disease processes in

CRC. Limitations include the finite number of SNPs, potential racial differences as the study used FinnGen data, and the need for larger sample sizes and further validation. Findings should be confirmed by RCTs and basic research.

5. Conclusion

Eight genetically proxied blood metabolites were found to have a causative influence on CRC in this MR study. Notably, Indolelactate, LPC (17:0), and LPC (18:0) warrant additional research as possible treatment targets for colorectal cancer. The identification of these serum metabolites offers important new information for the planning of upcoming clinical trials as well as for the early detection, prevention, and treatment of colorectal cancer. Furthermore, this MR analysis that combines metabolomics and genomes offers a guide for investigating the pathophysiology and etiology of CRC.

Supplementary materials: Table S1 covers 486 metabolites that we analyzed. **Table S2** shows the data results of 8 different analyses of the causal relationship between 486 blood metabolites and CRC by MR analysis. **Table S3** presents the results of IVW analysis of causality between 486 blood metabolites and CRC. **Table S4** shows the data results of co-localization analysis of 8 blood metabolites and CRC.

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