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Citric Acid Cycle Genetic Variants and Their Interactions with Obesity, Physical Activity and Energy Intake on the Risk of Colorectal Cancer : Results from a Nested Case-Control Study in the UK Biobank

시트르산 회로 단일 염기 다형성 및 환경요인 간 상호작용과 대장암 발생 위험 탐색

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Citric Acid Cycle Genetic Variants and Their Interactions with Obesity, Physical Activity and Energy Intake on the Risk of Colorectal Cancer : Results from a Nested Case-Control Study in the UK Biobank

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Abstract

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Colorectal cancer is one of the most common malignancies worldwide. Risk factors for the development of colorectal cancer include major contributors to energy balance, such as obesity and reduced physical activity. Based on these findings, physical activity, weight loss, and a healthy diet are recommended for the prevention of colorectal cancer. Even though there are individual differences in preventive effects, changes in lifestyle can affect cancer development with respect to metabolism in both the human body and cells. This study aimed to evaluate the association between genetic variants in the mitochondrial citric acid cycle and colorectal cancer to augment the explanation regarding individual differences in energy metabolism as genetic polymorphisms of mitochondria, which has a central

role in the energy metabolism at the cellular level. Interactions of single nucleotide polymorphisms (SNPs) in genes of the citric acid cycle with obesity, physical activity, and energy intake on colorectal cancer were also assessed. Furthermore, pairwise SNP-SNP interactions were examined to account for some missing heritability.

Data from the UK Biobank study were used. The study participants comprised of 3,523 colorectal cancer cases and matched 10,522 controls. Obesity was defined using body mass index (BMI) and waist-to-hip ratio (WHR). The participants were classified as obese if BMI is greater than or equal to 30 and severely obese if BMI is greater than or equal to 40. Participants with abdominal obesity were defined as men with a WHR > 0.9 and women with a WHR > 0.85. Participants who had excess energy intake were classified as having an estimated daily energy consumption of more than 2,000 kcal per day for women and 2,500 for men. Participants who performed over 150 minutes of moderate physical activity or 75 minutes of vigorous physical activity throughout the week were classified as those who achieved physical activity for general health benefits. The

main effects of the citric acid cycle SNPs were evaluated in the codominant, dominant, and additive models. Odds ratios (ORs) and 95% confidence intervals (95% CIs) for colon and rectal cancer were estimated using a conditional logistic regression model. The false discovery rate was used to correct multiple comparisons.

SUCLG2-rs35494829 was associated with a decreased risk of colon cancer in the dominant model (OR [95% CI]: 0.82 [0.74–0.92]) and additive model (0.82 [0.74–0.92]). The association between *SUCLG2*-rs35494829 and colon cancer was statistically significant after correcting for multiple comparisons (p=0.0206). The interaction between *SDHC*-rs17395595 and obesity for colon cancer was found ($p_{interaction}$=0.0023), and the significance of this interaction remained after correcting multiple comparisons (corrected $p_{interaction}$=0.047). Pairwise SNP-SNP interactions were also evaluated using the attributable proportion (AP) owing to interaction. Negative AP between the citric acid cycle SNPs for colon and rectal cancer with statistical significance is shown as follows. However, the P values did not reach statistical significance.

This study found a significant association between *SUCLG2*-rs35494829 and colon cancer. A significant interaction between *SDHC*-rs17395595 and obesity in colon cancer was also shown. This study evaluated the citric acid cycle SNPs, which were nonsynonymous SNPs or SNPs at a splicing site, as a functional candidate locus of the citric acid cycle in colorectal cancer. The findings in this study suggest that obesity could alter the association between variants in the citric acid cycle and colorectal cancer and may provide new insights into the genetic susceptibility and molecular mechanisms of obesity and the citric acid cycle on colorectal cancer.

Keywords: Colorectal Neoplasms; mitochondria; citric acid cycle; single nucleotide polymorphisms; obesity; physical activity

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1. Introduction

1.1. Colorectal cancer epidemiology

Colorectal cancer is commonly diagnosed worldwide. The GLOBOCAN estimated and reported the incidence of colorectal cancer; it is the third commonly occurring cancer observed among men and the second commonly observed cancer among women worldwide in 2018, with a geographic and ethnic variation [1].

In Korea, colorectal cancer was the second most common cancer reported in 2017 [2]. The age-standardized incidence rate for colorectal cancer is 32.0 per 100,000, and it increased by 5.9% annually from 1999–2010 and decreased by 4.2% annually from 2010–2017. The age-standardized incidence rate for this cancer is higher among men (ASR, 38.8 per 100,000) than that among women (ASR, 21.8 per 100,000).

Colorectal cancer in the UK

Colorectal cancer is also commonly reported in the UK. Colorectal cancer is the fourth most commonly observed cancer and it accounted for 11% of the new

cancer cases reported in 2017 (data available at https://www.cancerresearchuk.org/about-cancer/bowel-cancer) [3]. The European age-standardized rate is 55.2 per 100,000. Similar to the colorectal cancer incidence rate in Korea, the incidence rate in the UK is higher among men (rate [95% confidence intervals]; 83.2 [82.2–84.3] per 100,000) than that among women (68.0 [67.4–68.7] per 100,000). The number of new cases per year and age-specific incidence rates per 100,000 in the UK from 2015–2017 are shown in Figure 1. The age-specific incidence rates increased less remarkably until 85–89 of years in the age groups studied among both men (513.1 per 100,000) and women (356.2 per 100,000) and remained stable in most age groups (Figure 2). The incidence rates of colorectal cancer differed by ethnicity [4]. The White population (54.1–55.3 for men and 34.0–34.8 for women per 100,000) demonstrated the highest incidence rates of colorectal cancer, followed by the Black population (29.7–43.8 for men and 20.4–31.6 for women per 100,000) and Asian population (19.1–28.0 for men and 11.3–17.5 for women per 100,000) for both men and women.

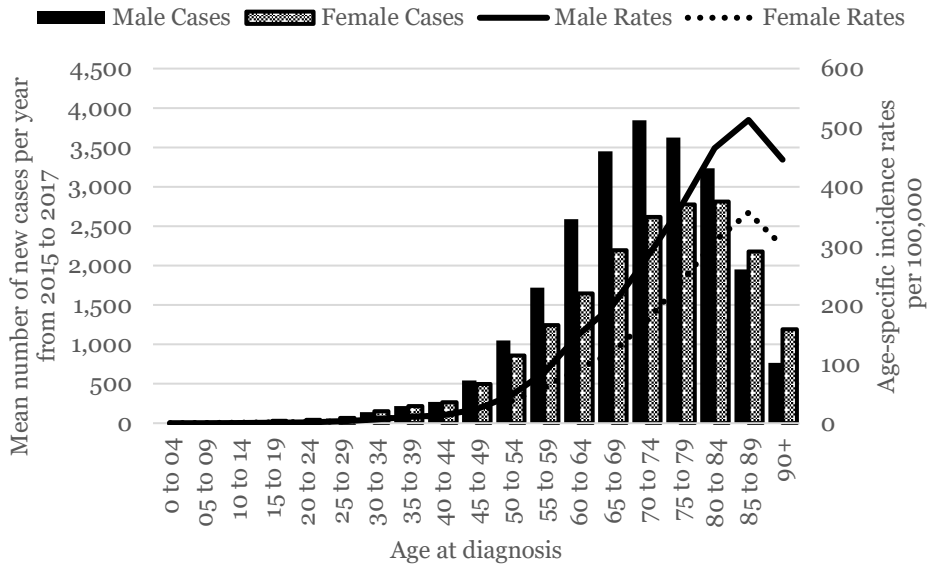


Figure 1. Number of new colorectal cancer cases per year and age-specific incidence rates per 100,000 in the UK from 2015–2017 [3].

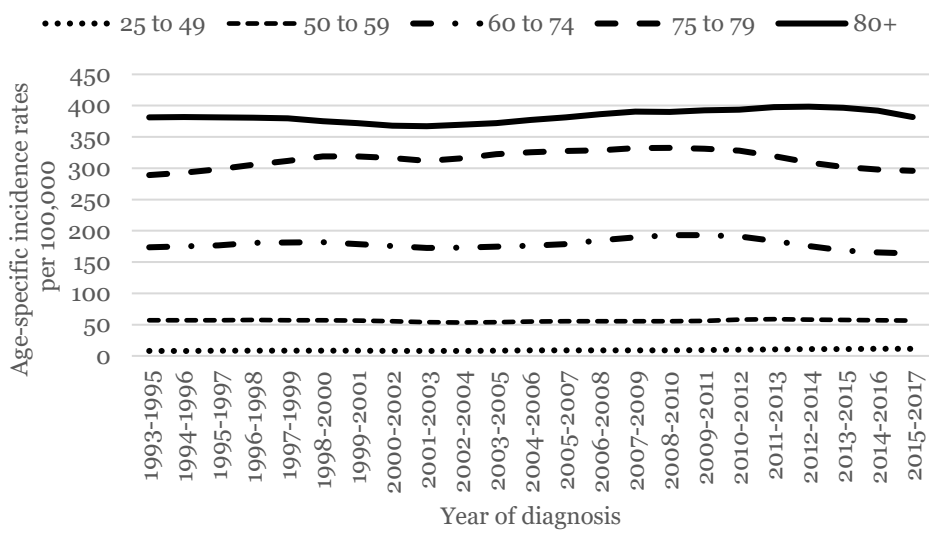


Figure 2. Age-specific incidence rates per 100,000 in the UK from 1993–2017 [3].

1.2. Well-known risk factors for colorectal cancer

1.2.1. Obesity

Obesity was considered a major risk factor for development of colorectal cancer with convincing evidence among both men and women in the cancer report published by the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) [5]. There was a difference observed in the effect of obesity on colorectal cancer risk according to sex, region, and anatomical subsite. The effect of size of obesity on colorectal cancer was more pronounced among men than women [5-7]. The Asian populations showed a higher risk of developing colorectal cancer incidence than the European population [6]. For the anatomical subsite, the association between obesity and colorectal cancer risk was remarkable in the colon than that in the rectum [5,6]. Not only general obesity, as identified by body mass index (BMI), but central obesity, as identified by waist circumference, is also related to increased risk of developing colorectal cancer [5,6].

1.2.2. Physical inactivity

WCRF/AICR reported that physical activity was associated with decreased

risk of developing colorectal cancer with "convincing" evidence [5]. A meta-analysis showed a statistically significant association between colorectal cancer risk and physical activity [8]. The protective effect of physical activity on colorectal cancer was significant in the colon, and not in the rectum [9,10], and was more remarkable among men than that in women [10], and among the participants with higher body mass index than those with lower body mass index [11]. Physical inactivity owing to insufficient participation in physical activity may be considered a risk factor for development of colorectal cancer. A meta-analysis on the association between sedentary behavior and increased colorectal cancer risk showed that the association was more pronounced in the colon than that in the rectum[12], and this was in concordance with the results reported by studies conducted on the relationship between physical activity and decreased colorectal cancer risk.

1.2.3. Energy intake

While diet is commonly considered an important factor for development of colorectal cancer in the context of other energy balance contributors, such as body size and physical activity, the association between energy intake and colorectal

cancer was not concluded owing to limited evidence in the WCRF/AICR report[5].

Results from previous studies were inconsistent with those on the effect of high energy consumption in colorectal cancer, compared with low consumption. High energy consumption associated with reduced risk of developing colorectal cancer was reported by a cohort study conducted in Finland [13] and the Singapore Chinese Health Study [14], while high energy consumption associated with increased risk of developing colorectal cancer was reported by the Women's Health Initiative study [15] and the Shanghai Women's Health Study [16]. The protective effect (relative risk, 0.90; 95% CI, 0.81–0.99) of high energy consumption, compared to low energy consumption, on colorectal cancer was reported by a meta-analysis [17].

1.3. Cell metabolism as a contributor to energy balance

Diet and physical activity are major contributors to maintenance of energy balance. Energy expenditure is categorized as resting energy expenditure and non-resting energy expenditure. Resting energy expenditure is defined as the energy expenditure through minimal metabolism required to perform basic body functions, and it is the largest component of energy expenditure [18]. Non-resting energy expenditure consists of exercise thermogenesis arising from exercise (physical activities), diet (ingestion, absorption, metabolism, and storage of nutrients from food), and non-exercise activities (energy expended during performance of non-exercise movements such as fidgeting or normal daily activities) [18]. Energy expenditure, including thermogenesis and basal metabolic rate, is closely associated with cell metabolism[19,20]. Mitochondria are the powerhouse in the cell and regulate their function according to the energy demands of the cell and prevalent conditions [21,22].

1.4. The mitochondria play a major role in energy metabolism

The mitochondria play a central role in energy metabolism. Part of the free energy derived from the oxidation of food inside the mitochondria is transformed into ATP, the energy currency of the cell. This process depends on oxygen consumption. Mitochondria are well appreciated as biosynthesis and bioenergetic organelles for their role in the production of metabolites and ATP, which are byproducts of the citric acid cycle and the mitochondrial membrane potential, respectively. In 1930, Warburg first reported mitochondrial somatic mutations in tumor cells and, in particular, suggested hypotheses on the abnormal function of the mitochondria and the development of cancer [23]. Based on the observation reported in 1956 [24], several studies have focused on mitochondrial impairment related to altered respiratory pathways of energy metabolism in the development of cancer. The efficiency to produce energy from a substrate, defined as metabolic rate, varies within species as well as between species [25]. Although presence of intra-specific variations can be explained by species-specific characteristics of the

mitochondria [26], inter-specific variations can be described by the mode of temperature regulation, body-size range, and activity levels [27].

1.5. Mitochondrial citric acid cycle as a biomarker for cancer

The citric acid cycle plays a central role in cellular energy metabolism and the biosynthesis of macromolecules through a series of biochemical reactions, which occur in the mitochondrial matrix. It has been hypothesized that the abnormal function of the citric acid cycle can lead to the development of pathological conditions. An *in vitro* study reported a significant association between the intermediates of the citric acid cycle and the regulation of hypoxia-inducible factor (HIF), which is a transcription factor involved in angiogenesis, glucose utilization, and apoptosis [28]. The activity of citric acid cycle enzymes, including citric synthase, are reduced in mice under the nutrient-excess conditions [29]. Tumor cells separate processes from the citric acid cycle, allowing them to respond to elevated metabolic levels using additional energy sources, such as glutamine, which was established as essential nutrient sources in the development of various types of cancers [30].

1.6. Previous studies on the interaction of obesity, physical activity, and energy intake with genetic factors on cancer risk and SNP-SNP interaction in colorectal cancer

PubMed was used to explore previous studies on the interaction of obesity, physical activity, and energy intake with genetic factors on cancer risk and SNP-SNP interaction in colorectal cancer. Previous studies on the interaction of environmental factors (E), including obesity, physical activity, and energy intake, with genetic factor (G) in cancer, were found by using the following MeSH keywords as follows: "Gene-Environment Interaction"[MeSH] AND "polymorphism, single nucleotide"[MeSH] AND ("obesity" OR "physical activity" OR "energy intake") AND "Neoplasms"[MeSH] AND "risk"[MeSH]. Nineteen articles were found, and five articles were excluded, which were not original articles (*n*=1), not based on evaluated interaction with the desirable environmental factors (*n*=2), or not based on cancer (*n*=2). Thereafter, the remaining 14 articles were reviewed.

Previous studies on SNP-SNP interactions in colorectal cancer were explored

using the following MeSH keywords: (snp-snp interaction[Title/Abstract] OR gene-gene interaction[Title/Abstract]) AND ”cancer, colorectal”[MeSH Terms] AND human[MeSH Terms] AND polymorphisms, single nucleotide[MeSH Terms]. Eight articles were found and reviewed with the exception of an article[31], which was not available in the full-text format[32-38].

1.6.1. Previous studies on the interaction of obesity, physical activity, and energy intake with genetic factors in cancer

Understanding the mechanisms by which obesity, physical activity, and energy intake are associated with genetic factors and modify cancer incidence can be supported by studies on gene-environmental (G×E) interactions. A previous study reported in the Women's Health Initiative Database for Genotypes and Phenotypes Study (WHI dbGaP) evaluated the effect of insulin resistance, which was genetically attributed to the gene expression levels in response to the levels of fasting glucose, fasting insulin, and homeostatic model assessment-insulin resistance, and the interactions with obesity and physical activity to assess the risk

of developing breast cancer in postmenopausal women, and reported that participants who were classified as overall obesity or inactive subgroups exhibited a greater risk of developing breast cancer [39]. Another study in the WHI dbGAP reported that *NR5A2* rs10919774, which was an index SNP of hyperinsulinemia, was associated with decreased risk of developing breast cancer in participants with BMI values under 30 kg/m^2, although this association was not observed in participants with a BMI equal to or more than 30 kg/m^2 [40]. Another study reported in the WHI dbGAP evaluated the combined effects of the SNPs related to insulin resistance and behavioral factors on the risk of developing colorectal cancer and reported an 8-fold increased risk for colorectal cancer development in the participants of both studies who harbored risk alleles and who were in the physically active groups [41].

Table 1 presents previous studies on the interaction between obesity and genetic factor conducted on the risk of cancer development for several cancer sites, including esophageal cancer [42], pancreatic cancer [43,44], prostate cancer risk [45], breast cancer [46], and ovarian cancer [47,48]. A study using data reported by the International

Barrett's Esophagus and Adenocarcinoma Consortium (BEACON) evaluated the interaction of smoking, obesity, reflux, and NSAID use with 7,863 SNPs in 449 genes related to five pathways (cyclooxygenase [COX], cytokine signaling, oxidative stress, human leucocyte antigen, and nuclear factor-κB) for esophageal adenocarcinoma and Barrett's esophagus, which are the precursors of esophageal adenocarcinoma, but there were no significant G×E interactions observed for esophageal adenocarcinoma and Barrett's esophagus [42]. Significant interactions of obesity with pancreatic cancer have been presented with the SNPs which were assigned to the chemokine signaling pathway in the study using the data reported by the Pancreatic Cancer Case Control Consortium [43]; *PDX1* rs9581943 of previously identified pancreatic cancer SNPs shared a statistically significant association with obesity in a hospital-based case-control study among Taiwanese [44].

The interaction of obesity with the genetic factor for prostate cancer was evaluated with the SNPs in estrogen-related pathway genes, including *ESR1*, *ESR2*, *CYP19A1*, *CYP1A1*, and *CYP1B1*, in a population-based case-control study consisting of Caucasian men and significant G×E interactions were not observed [45].

Interactions of obesity to assess the risk of breast cancer development were explored using the SNPs in the adiponectin gene and leptin gene, which were previously reported to influence plasma levels of adiponectin and leptin, in a prospective case-control study in south India, and there was no significant interaction [46].

A study on the interaction of obesity with ovarian neoplasms among the participants in 14 case-control studies (6,247 cases; 10,379 controls), which were part of the Collaborative Oncological Gene Environment Consortium, was conducted using 11,441 SNPs within 80 genes related to oral contraceptive use, parity, endometriosis, tubal ligation, hormone replacement therapy, and estrogen use, and a significant interaction between obesity and *INSR* rs113759408 related to parity was reported for endometrioid ovarian cancer [47]. The interaction of obesity with *PIK3CA* rs2699887, rs3976507, and rs6443626 has been also explored to assess the risk of developing ovarian cancer in the Chinese Han population and the interaction between *PIK3CA* rs3976507 and rs6443626, and BMI to assess the risk of developing ovarian cancer [48].

Previous studies on the interaction between physical activity and genetic factors have been conducted to assess the risk of developing breast cancer [40,49] and colorectal cancer [41] (Table 2). Interaction of variations in *CYP27B1* and the microRNA-binding site of *IL-13* with regular physical activity were evaluated and a significant interaction was observed between physical activity ≥1 time per week and rs10877012 and rs4646536 in CYP27B1 [40]. SNPs associated with insulin phenotypes (*MTRR* rs722025, *MKLN1* rs117911989) showed significant interactions with physical activity (active group [MET≥10; inactive group [MET<10]) for colorectal cancer development [41], while there were no significant interactions observed between SNPs associated with insulin resistance phenotype and physical activity [49] for breast cancer development. Although studies on the interaction of total energy intake with genetic factors are not available, the interaction between SNPs associated with insulin resistance with an intake of dietary fat (defined as the percentage of calories from saturated fatty acids) has been reported [41] (Table 3).

1.6.2. Previous studies on SNP-SNP interactions in colorectal cancer

Table 4 shows a summary of previous studies on G×G interactions in colorectal cancer. Polymorphisms in xenobiotic-metabolizing enzymes (*CYP1A1* c.1384A>G and *EPHX1* c.337T>C) showed significant interactions in colorectal cancer development [32]. The results from a genome-wide study to determine pairwise G×G interactions have shown the interactions between rs10795668 and rs367615, and rs1571218 and rs10879357 [33]. A hospital-based case-control study suggested that the functional variations in murine double minute 2 protein (MDM2) and TP53 might lead to colorectal cancer susceptibility, and showed significant interactions between *TP53* Arg72Pro and *MDM2* T309G among only smokers [34]. G×G interactions were also evaluated for polymorphisms of the insulin resistance genes, including adiponectin (ADIPOQ) rs2241766, uncoupling protein 2 (UCP2) rs659366, and fatty acid-binding protein (FABP2) rs1799883, and a significant interaction was observed between *ADIPOQ* rs2241766 and *FABP2* rs1799883 [35]. The interactions of SNPs in mismatch repair genes (*hMLH1* and

hMSH2) have been assessed, and the three-way gene-gene interactions of IVS11+107A>G, IVS11+183A>G, and IVS8+719A>G were found to be significant [36]. A case-control study reported by the Korean Cancer Prevention study-II cohort has evaluated the G×G interactions of T-cadherin, which has been identified as adiponectin receptor and is associated with adiponectin levels, and reported the significant interactions of rs3865188 with rs2241767, rs3821799, rs3774261, and rs6773957 [37]. Other G×G interactions were evaluated for interleukin-12, which is an antitumor cytokine, and there were significant interactions observed between *IL-12A* rs568408 and *IL-12B* rs3212227, and *IL-12A* rs568408 and *IL-12B* rs3212227 [38].

Table 1. Previous studies on interactions of genetic factors with obesity for cancer risk.

Reference	Genetic factor	Outcome	Results G main effect	Results GxE interaction effect
Holt, S.K., et al., Prostate, 2013. 73(1): p. 1-10.	Estrogen-related pathway genes	Prostate cancer	Found altered risk for variants in ESR1, CYP1A1, and CYP1B1, but only CYP1B1 rs1056836 remained significance after adjustment for multiple comparisons.	No effect modification by obesity
Tang, H., et al., Cancer Epidemiol Biomarkers Prev, 2014. 23(1): p. 98-106.	Genome-wide	Pancreatic cancer	-	Significant interaction of the chemokine signaling pathway with obesity ($P = 3.29 \times 10\text{-}6$)
Shan, Y.S., et al., J Biomed Sci, 2020. 27(1): p. 69.	25 pancreatic cancer SNPs identified from previous GWAS	Pancreatic cancer	NR5A2 rs2816938, MYC rs10094872, PDX1 rs9581943 and 4 chromosome 13q22.1 SNPs: rs4885093, rs9573163, rs9543325, rs9573166	PDX1 rs9581943 with obesity
Buas, M.F., et al., Gut, 2017. 66(10): p. 1739-1747.	Variants of inflammation-related pathways	Oesophageal adenocarcinoma	MGST1 variants influence Oesophageal adenocarcinoma susceptibility.	No statistically significant interactions

Table 1. Continued

Reference	Genetic factor	Outcome	Results G main effect	Results GxE interaction effect
Li, H., et al., Am J Epidemiol, 2013. 177(2): p. 161-70.	Cumulative genetic risk score, constructed from 10 variants with replicated associations	Breast cancer	-	No interaction of genetic risk score with obesity
Geriki, S., et al., Mol Biol Rep, 2019. 46(6): p. 6287-6297.	SNPs of adiponectin and leptin genes	Breast cancer	Adiponectin rs1501299 and leptin rs7799039	No significant interaction
Jung, S.Y., et al., PLoS One, 2019. 14(6): p. e0218917.	Genetically driven insulin resistance using Mendelian randomization	Breast cancer	Genetically elevated fasting glucose was associated with reduced risk for breast cancer	Greater breast cancer risk in overall obesity
Jung, S.Y., et al., Cancer Prev Res (Phila), 2019. 12(1): p. 31-42.	SNPs associated with insulin resistance phenotype	Breast cancer	29 were associated with postmenopausal breast cancer	Significant interactions between NR5A2 rs10919774 and obesity

Table 1. Continued

Reference	Genetic factor	Outcome	Results G main effect	Results GxE interaction effect
Usset, J.L., et al., Cancer Epidemiol Biomarkers Prev, 2016. 25(5): p. 780-90.	Genes- related to hormone bio synthesis and metabolism and insulin-like growth factor	Ovarian cancer	-	Notable obesity–gene–hormone risk factor interaction was within INSR rs113759408 (P¼ 8.8 x10-6)
Zhang, H. and L. Zhou, Pathol Res Pract, 2019. 215(9): p. 152520.	PIK3CA rs2699887, rs3976507, rs6443626	Ovarian cancer	PIK3CA rs2699887	Rs3976507 and rs6443626 with obesity

Table 2. Previous studies on interactions of genetic factors with physical activity for cancer risk.

Reference	Genetic factor	Outcome	Results G main effect	Results GxE interaction effect
Li, H., et al., Am J Epidemiol, 2013. 177(2): p. 161-70.	Cumulative genetic risk score, constructed from 10 variants with replicated associations	Breast cancer	-	No significant interaction of genetic risk score with regular physical activity
Nickels, S., et al., PLoS Genet, 2013. 9(3): p. e1003284.	Common Breast Cancer Susceptibility Loci	Breast cancer	Rs11249433, CASP8 rs17468277, rs13387042, SLC4A7 rs4973768, rs10941679, MAP3K1 rs889312, ESR1 rs12662670, ESR1 rs2046210, rs13281615, CDKN2A/B rs1011970, rs865686, ZNF365 rs10995190, ZMIZ1 rs704010, FGFR2 rs2981582, rs614367, LSP1 rs3817198, PTHLH rs10771399, rs1292011, RAD51L1 rs999737, TOX3 rs380366,2 COX11 rs6504950, NRIP1 rs2823093	No significant interaction

Table 2. Continued.

Reference	Genetic factor	Outcome	Results G main effect	Results GxE interaction effect
Zhang, N., et al., Cancer Med, 2019. 8(6): p. 3237-3249.	Variations in CYP27B1 and the microRNA-binding site of IL-13	Breast cancer	-	≥1 time/week physical activity with rs10877012 and rs4646536 in CYP27B1
Jung, S.Y., et al., Cancer Prev Res (Phila), 2019. 12(1): p. 31-42.	SNPs associated with insulin resistance phenotype	Breast cancer	29 were associated with postmenopausal breast cancer	No significant interaction
Jung, S.Y., et al., Cancer Prev Res (Phila), 2019. 12(12): p. 877-890.	SNPs associated with insulin phenotype resistance using random survival forest analysis a machine learning method	Colorectal cancer	LINC00460 rs1725459 and MTRR rs722025	MTRR rs722025, MKLN1 rs117911989 with physical activity(active group[MET≥10; inactive group [MET<10])

Table 3. Previous studies on interactions of genetic factors with energy intake for cancer risk.

Reference	Genetic factor	Outcome	Results G main effect	Results GxE interaction effect
Jung, S.Y., et al., Cancer Prev Res (Phila), 2019. 12(12): p. 877-890.	SNPs associated with insulin phenotype resistance using random survival forest analysis a machine learning method	Colorectal cancer	LINC00460 rs1725459 and MTRR rs722025	LINC00460 rs17254590 with dietary-fat intake(percentage of calories from saturated fatty acids)

Table 4. Previous studies on GxG interactions for colorectal cancer

Reference	Genetic factor	Results G main effect	Results GxG interaction effect
Pande, M., et al., Mol Carcinog, 2010. 49(11): p. 974-80.	CYP1A1 c.1384A>G and EPHX1 c. 337T>C . Polymorphisms in xenobiotic metabolizing enzymes	-	CYP1A1 c.1384A>G and EPHX1 c.337T>C
Jiao, S., et al., PLoS One, 2012. 7(12): p. e52535.	Genome-Wide Search for pairwise GxG	-	Rs10795668 and rs367615, rs1571218 and rs10879357
Zhang, Y., et al., Mol Biol Rep, 2012. 39(10): p. 9661-8.	TP53 Arg72Pro and MDM2 T309G	Not significant	TP53 Arg72Pro and MDM2 T309G among smokers
Hu, X., et al., PLoS One, 2013. 8(6): p. e67275.	Insulin resistance-related gene polymorphisms of adiponectin (ADIPOQ) rs2241766, uncoupling protein 2 (UCP2) rs659366, and fatty acid-binding protein (FABP2) rs1799883	Rs2241766 in dominant model	Rs2241766 and rs1799883

Table 4. Continued

Reference	Genetic factor	Results G main effect	Results GxG interaction effect
Li, G., et al., J Cancer Res Clin Oncol, 2015. 141(8): p. 1393-404.	Intronic and promoter polymorphisms of hMLH1/hMSH2	IVS11+107A>G and IVS8+719T>C for colon cancer in dominant model	IVS11+107A>G, IVS11+183A>G and IVS8+719A>Ga
Park, J., et al., J Biomed Sci, 2015. 22(1): p. 73.	Polymorphisms of T-cadherin gene (CDH13 and APN)	CDH1 rs3865188 in recessive model	Rs3865188 with rs2241767, rs3821799, rs3774261,and rs6773957
Sun, R., et al., Tumour Biol, 2015. 36(12): p. 9295-301.	IL-12A rs568408, IL-12A rs2243115, and IL-12B rs3212227	IL-12A rs568408 in dominant model	IL-12A rs568408 and IL-12B rs3212227; IL-12A rs568408 and IL-12B rs3212227

2. Research objectives

The study aimed to assess the polymorphism of the mitochondrial citric acid cycle colorectal cancer and to examine the possible interactions between SNPs in the citric acid cycle and the contributors to energy balance, including obesity, physical activity, and energy intake. Furthermore, it was suggested that pairwise SNP interactions of the citric acid cycle might exert effects on cancer because of the nature of the cycle. SNP-SNP interactions of pairwise SNPs in the citric acid cycle in colorectal cancer were also examined.

3. Materials and methods

3.1. Study population

The sample used for this study consisted of individuals who participated in the UK Biobank study. UK Biobank is a national resource, initially developed to study lifestyle and genetic factors affecting aging traits with the aim of understanding and improving healthy aging at the population level. Participants were registered with the UK National Health Service and resided within 25 miles of one of the 22 assessment centers. More than 500,000 volunteers were enrolled across the UK between 2007 and 2013, and they donated samples for genotyping, completed lifestyle questionnaires, and were subjected to tests for standard measurements. The UK Biobank resource is described extensively elsewhere [50,51].

3.2. Data collection and measurements

Primary interest in environmental exposure

Body size, including waist and hip circumference, height, and weight, was directly measured at enrolment. Obesity was defined using BMI values and waist to hip ratio (WHR). The participants were classified and people with over or equal to 30 kg per m^2 of BMI were defined as individuals with obesity and the participants with over or equal to 40 kg per m^2 of BMI as were defined as individuals with severe obesity. Waist to hip ratio was also used to assess obesity. Men with over 0.9 of WHR and women with over 0.85 were classified as the participants with abdominal obesity.

Energy intake was estimated as consumption of nutrients via diet by 24-h recall and results with the units in KJ were reported. The estimated amount of daily energy consumption was more than 2,000 kilocalories a day for women and 2,500 kilocalories for men, which were classified as excess energy intake. The participants also reported information on physical activities, including the number of days per week of moderate/vigorous physical activity more than 10 min and the

duration of moderate/vigorous activity on a typical day. Participants who performed over 150 min of moderate physical activity or 75 min of vigorous physical activity throughout the week were classified as individuals who achieved the physical activity necessary for experiencing general health benefits.

Table 5. Definition of exposures.

Classification	Exposure	Category
Obesity	Obesity, BMI	<30 kg/m^2 ≥30 kg/m^2
	Severe obesity, BMI	<40 kg/m^2 ≥40 kg/m^2
	Abdominal obesity, WHR	≤0.9 for men and ≤0.85 for women >0.9 for men and >0.85 for women
Physical activity	Moderate physical activity	Not sufficient (participants who performed less than 150 minutes of moderate physical activity throughout the week) Sufficient (participants who performed over 150 minutes of moderate physical activity throughout the week)
	Vigorous physical activity	Not sufficient (participants who performed less than 75 minutes of vigorous physical activity throughout the week) Sufficient (participants who performed over 75 minutes of vigorous physical activity throughout the week)
	Moderate or vigorous physical activity	Not sufficient (participants who performed neither 150 minutes of moderate physical activity nor 75 minutes of vigorous physical activity) Sufficient (participants who performed over 150 minutes of moderate physical activity or 75 minutes of vigorous physical activity)
Energy intake	Daily energy intake, kcal	<2000 kcal for men and <2500 kcal for women <2000 kcal for men and ≥2500 kcal for women

Matching variable

Information on ethnicity was collected at enrolment. Participants reported their ethnic group, and were classified as follows (Table 6): British, Irish, or any other White background was classified as White; mixed ethnicity background between White and Black Caribbean, White and Black African, White and Asian or any other mixed background was classified as mixed; Indian, Pakistani, Bangladeshi or any other Asian background was classified as Asian or Asian British; Caribbean, African or any other Black background was classified as Black or Black British; Chinese; and others.

Table 6. Classification of ethnic background.

Classification	Ethnic group
White	British, Irish or any other white background
Mixed	Mixed ethnic background between White and Black Caribbean, White and Black African, White and Asian or any other mixed background
Asian or Asian British	Indian, Pakistani, Bangladeshi or any other Asian background
Black or Black British	Caribbean, African or any other Black background
Chinese	-
Other	-

Townsend deprivation index scores were derived from the national census data. This index was calculated based on four variables as follows: car ownership, household overcrowding, owner-occupation, and unemployment aggregated for postcodes of residence[52]. Higher Townsend scores were equated to higher levels of socioeconomic deprivation. The data on household income were self-reported. The Townsend deprivation index was categorized by quartile among both the control and the cases included in the analyses.

3.3. Outcome ascertainment

Data on cancer diagnoses and deaths were obtained by using the UK Biobank through the National Health Service (NHS) Digital for participants in England and Wales and the NHS Central Register for participants in Scotland. The completeness of the case ascertainment in English cancer registries is reported to be approximately 98%–99%, based on a study that linked routine cancer registration with information from the Hospital Episode Statistics database [53]. Cancers of the colon and rectum were classified according to the 10th revision of the International Classification of Diseases (ICD-10; C18, and C19-C20, respectively), only for cancer diagnosed after enrolment.

3.4. Case and control selection

A total of 502,536 participants were subjected to follow-up until December 2016. A total of 483,149 participants remained after the exclusion of participants who presented no information on cancer incidence, genotype, ethnic background, and socioeconomic deprivation, and 3,637 participants were identified as the incident cases of colorectal cancer. For each case, three controls were selected using incidence density sampling [54] from participants who were diagnosed with colorectal cancer with matching performed based on sex, age group at enrolment by 5 years, ethnic background (White, Mixed, Asian or Asian British, Black or Black British, Chinese, and Others). There were 22 study centers (Barts, Birmingham, Bristol, Bury, Cardiff, Croydon, Edinburgh, Glasgow, Hounslow, Leeds, Liverpool, Manchester, Middlesbrough, Newcastle, Nottingham, Oxford, Reading, Sheffield, Stockport, Stoke, Swansea, Wrexham) and Townsend deprivation index at recruitment (divided to four groups by quartiles). Figure 3 shows the flow chart of case and control selection of data obtained from the UK biobank.

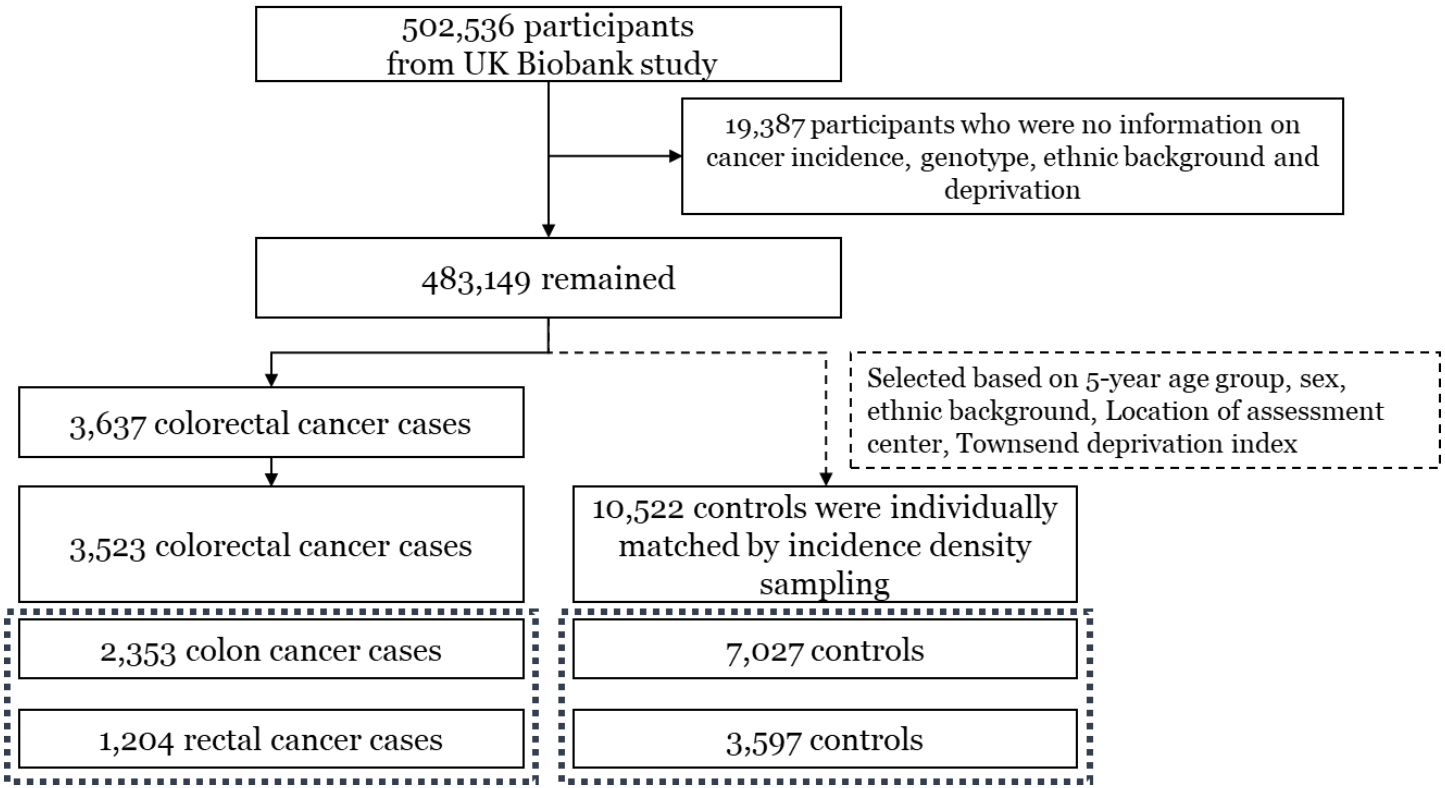


Figure 3. Flow chart of case and control selection in data from the UK biobank.

3.5. Genotyping

Participants answered detailed questions about themselves, were subjected to measurement tests, and provided blood, urine, and saliva samples. Two arrays with over 95% common marker content were used for genotyping of the individuals. The UK Biobank data release available at the time of analysis included genotypes for 488,377 participants, obtained through either the custom UK Biobank Axiom array or the Affymetrix Axiom Array. The genotypes were imputed to the Haplotype Reference Consortium 48, and the combined UK10K/1000 Genomes panels were retrieved from the UK Biobank data showcase [55].

3.6. Marker selection

The MitoProteome database (available at http://www. mitoproteome.org/) was used to select the genes contributing to the citric acid cycle [56]. The citric acid cycle genes were selected based on analysis using the Kyoto encyclopedia of genes and genomes (KEGG) [57] and the keyword of "Citrate cycle (TCA cycle)"; data on 27 autosomal genes were extracted (Table 7). Then, data on the SNPs within the 27 genes related to the TCA (tricarboxylic acid) cycle were found using the dbSNP database [38]. SNPs related to the citric acid cycle were selected based on the following criteria: 1) genetic variant of the mitochondrial citric acid cycle; 2) functionally important variant that might affect gene transcript structure or protein, such as coding nonsynonymous SNPs or SNPs at a splicing site; 3) common variant allele with minor allele frequency (MAF) > 5%; and 4) genotype call rate > 99%. Among the 24 selected SNPs, rs16832869 in the *SDHC* gene, rs2303436 in the *DLAT* gene, rs751595 in the *OGDHL* gene were excluded owing to remarkable linkage disequilibrium with an r^2 over 0.6. Finally, the 21 citric acid cycle SNPs were included in the analyses. Figure 4 shows the strategy for marker selection.

Table 8 describes the information on SNPs, which met the inclusion criteria.

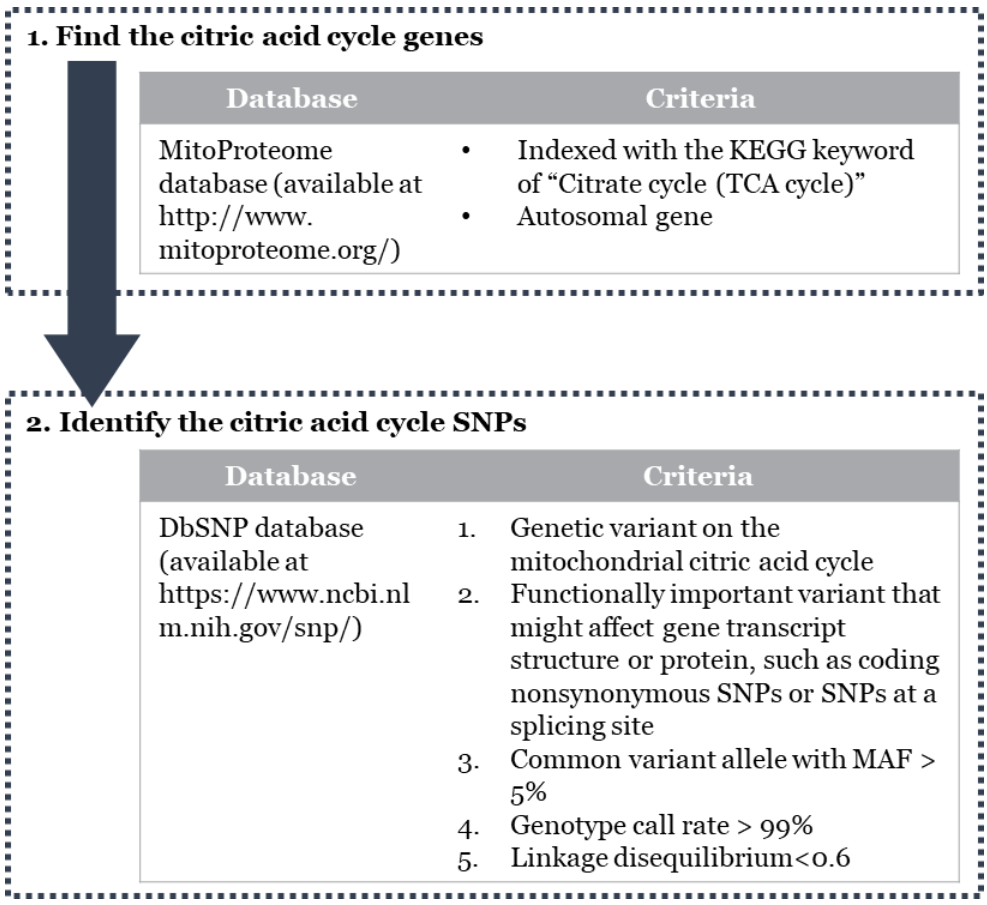


Figure 4. Marker selection strategy

Table 7. Nuclear-encoded mitochondrial proteins within the citric acid cycle

Symbol	Description	Location	Start	End	Orientation	Exon count	OMIM
SDHB	succinate dehydrogenase complex iron sulfur subunit B	1p36.13	17018722	17054032	minus	8	185470
SDHC	succinate dehydrogenase complex subunit C	1q23.3	161314381	161375340	plus	7	602413
FH	fumarate hydratase	1q43	241497603	241519755	minus	10	136850
MDH1	malate dehydrogenase 1	2p15	63588963	63607197	plus	10	154200
SUCLG1	succinate-CoA ligase GDP/ADP-forming subunit alpha	2p11.2	84423528	84459280	minus	9	611224
IDH1	isocitrate dehydrogenase (NADP(+)) 1	2q34	208236227	208255071	minus	12	147700
PDHB	pyruvate dehydrogenase E1 subunit beta	3p14.3	58427630	58433852	minus	9	179060
SUCLG2	succinate-CoA ligase GDP-forming subunit beta	3p14.1	67360460	67654614	minus	14	603922
PDHA2	pyruvate dehydrogenase E1 subunit alpha 2	4q22.3	95840093	95841464	plus	1	179061
SDHA	succinate dehydrogenase complex flavoprotein subunit A	5p15.33	218223	264816	plus	15	600857
OGDH	oxoglutarate dehydrogenase	7p13	44606572	44709066	plus	26	613022
MDH2	malate dehydrogenase 2	7q11.23	76048106	76067508	plus	10	154100
DLD	dihydrolipoamide dehydrogenase	7q31.1	107891107	107921198	plus	14	238331
ACO1	aconitase 1	9p21.1	32384603	32454769	plus	23	100880
OGDHL	oxoglutarate dehydrogenase like	10q11.23	49734641	49762379	minus	24	617513

OMIM, Online Mendelian Inheritance in Man database

Table 7. Continued.

Symbol	Description	Location	Start	End	Orientation	Exon count	OMIM
PC	pyruvate carboxylase	11q13.2	66848420	66958418	minus	32	608786
DLAT	dihydrolipoamide S-acetyltransferase	11q23.1	112025408	112064404	plus	14	608770
SDHD	succinate dehydrogenase complex subunit D	11q23.1	112086873	112095794	plus	6	602690
CS	citric synthase	12q13.3	56271699	56300330	minus	11	118950
SUCLA2	succinate-CoA ligase ADP-forming subunit beta	13q14.2	47942656	48001273	minus	11	603921
PCK2	phosphoenolpyruvate carboxykinase 2, mitochondrial	14q11.2-q12	24094311	24104125	plus	10	614095
DLST	dihydrolipoamide S-succinyltransferase	14q24.3	74881913	74903743	plus	15	126063
IDH3A	isocitrate dehydrogenase (NAD(+)) 3 catalytic subunit alpha	15q25.1	78149362	78171945	plus	12	601149
IDH2	isocitrate dehydrogenase (NADP(+)) 2	15q26.1	90083045	90102468	minus	12	147650
ACLY	ATP citric lyase	17q21.2	41866916	41930542	minus	30	108728
IDH3B	isocitrate dehydrogenase (NAD(+)) 3 non-catalytic subunit beta	20p13	2658394	2664223	minus	14	604526
ACO2	aconitase 2	22q13.2	41468756	41528979	plus	19	100850

OMIM, Online Mendelian Inheritance in Man database

Table 8. Information on the 21 citric acid cycle SNPs which were included in the present study.

Gene	SNP	Chr:position	Allele (a<A)	MAF		*p* for HWE	Call rate (%)
				Control	CRC Case		
SDHC	rs16832884	1:161368670	G<A	0.061	0.063	0.883	99.7
SDHC	rs17395595	1:161374656	G<A	0.148	0.147	0.788	99.9
MDH1	rs2278718	2:63588667	C<A	0.249	0.244	0.365	99.8
IDH1	rs34218846	2:208243593	T<C	0.056	0.054	1.000	99.7
SUCLG2	rs902320	3:67360679	T<C	0.270	0.261	0.451	99.9
SUCLG2	rs902321	3:67360742	G<A	0.395	0.389	0.296	99.8
SUCLG2	rs35494829	3:67375857	C<T	0.113	0.101	0.829	99.9
SUCLG2	rs2363712	3:67376176	T<C	0.327	0.317	0.289	99.9
SDHA	rs6962	5:256394	A<G	0.129	0.129	0.099	99.9
SDHA	rs34511054	5:264041	C<A	0.059	0.061	0.651	99.8
ACO1	rs7042042	9:32451146	A<G	0.356	0.356	0.740	99.9
ACO1	rs10970986	9:32453280	C<T	0.291	0.294	0.919	99.9
OGDHL	rs11101224	10:49742930	A<G	0.179	0.179	0.096	99.7
DLAT	rs10891314	11:112045923	A<G	0.368	0.349	0.570	99.9
PCK2	rs55733026	14:24095963	G<A	0.074	0.068	1.000	99.2
PCK2	rs1951634	14:24100525	T<G	0.254	0.252	0.738	99.9
PCK2	rs35618680	14:24103603	A<G	0.090	0.088	0.796	99.1
IDH3A	rs11555541	15:78149427	C<T	0.495	0.495	0.418	99.9
IDH3A	rs17674205	15:78169115	G<A	0.089	0.084	0.833	100.0
ACLY	rs8065502	17:41892360	A<G	0.085	0.085	0.355	99.6
ACLY	rs2304497	17:41909521	G<T	0.125	0.126	0.232	99.9

CRC denotes colorectal cancer, SNP denotes single nucleotide polymorphism, MAF denotes minor allele frequency, HWE denotes Hardy-Weinberg equilibrium.

P-values were calculated using Pearson′s χ^2 tests; A and a were designated as the major and minor alleles, respectively.

3.7. Statistical analysis

Conditional logistic regression was used to estimate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of SNPs related to the citric acid cycle assuming the additive and dominant model of colorectal cancer by subsites with the adjustment of the smoking and alcohol consumption status. The deviation from the Hardy–Weinberg equilibrium (HWE) among the controls was assessed using a Pearson's chi-squared test. *P* for interaction was calculated using the likelihood ratio test. Stratified analyses were also conducted using the number of minor alleles only when the interaction between p-values was under 0.05. Genotypes of the SNP were dichotomized to noncarrier and carrier of the minor allele in the analyses of the gene-environment interactions and the SNP-SNP interactions.

Pairwise SNP-SNP interactions were evaluated using the relative excess risk due to interaction (RERI) and the attributable proportion due to interaction (AP) [58]. The SNPs were dichotomized, assuming the dominant model in the analyses of gene-environment and SNP-SNP interactions. RERI describes the effects arising

due to interactions between two dichotomous risk factors, calculated with the following formula. :

$$\mathrm{RERI} = \mathrm{RR}_{\mathrm{E1+E2+}} - \mathrm{RR}_{\mathrm{E1+E2-}} - \mathrm{RR}_{\mathrm{E1-E2+}} + 1$$

RR_{E1+E2+}: relative risks for the presence of both exposure1 and exposure2

RR_{E1+E2-}: relative risks for the presence of exposure1 and the absence of exposure2

RR_{E1-E2+}: relative risks for the absence of exposure1 and the presence of exposure2

AP indicates the measure quantifying the proportion of the combined effect due to interaction.

$$\mathrm{AP} = \frac{RERI}{RR_{E1+E2+}}$$

RERI: relative excess risk due to interaction

RR_{E1+E2+}: relative risks for the presence of both exposure1 and exposure2

The value of AP ranged from -1 to +1. An AP greater than zero indicates a positive interaction or more than additivity. An AP of less than zero indicates a negative interaction or less than additivity. The 95% CIs of AP were calculated

using the delta method, which was described by Hosmer and Lemeshow [59]. It is recommended that the risk factors rather than the preventive factors should be considered when calculating RERI and AP [60]. Therefore, if the main effect of the SNP was preventive (that is, OR<1), carrier of minor allele was considered as the reference category in the analyses of the SNP-SNP interactions.

Two-sided *p*-values less than 0.05 were regarded as statistically significant. Bold font indicates statistical significance with a p-value of less than 0.05. The statistical analyses were conducted using the R software version 3.6.3. Pairwise linkage disequilibrium was assessed using the Haploview software version 4.2 [61]. LocusZoom was used to generate plots for regional visualization of the results [62]. The P-values were adjusted using the false discovery rate (FDR) for multiple comparisons, proposed by Benjamini and Hochberg [63].

4. Results

4.1. Characteristics of participants

Table 9 summarizes selected baseline characteristics of matched variables by cases and controls. A total of 10,522 controls and 3,523 cases were included in the analyses. Participants aged 61–65 years (n [%]; 3,361 [31.9%] in controls and 1,123 [31.9%] in cases) were the most common, followed by 66–70 (3,046 [28.9%] in controls and 1,021 [29.0%] in cases), 56–60 (2,007 [19.1%] in controls and 412 [11.7%] in cases), 51–55 (1,237 [11.8%] in controls and 673 [19.0%] in cases), 46–50 (575 [5.5%] in controls and 193 [5.5%] in cases), 41–45 (272 [2.6%] in controls and 93 [2.6%] in cases), and 36–40 (24 [0.2%] in controls and 8 [0.2%] in cases) at enrollment. The participants included more men (6,052 [57.5%] in controls and 2,024 [57.5%] in cases) than women (4,470 [42.5%] in controls and 1,499 [42.5%] in cases).

Most of the study participants were White (10,284 [97.7%] in controls and 3,423 [97.2%] in cases), followed by Asian or Asian British (83 [0.8%] in controls and 31 [0.9%] in cases), Black or Black British (76 [0.7%] in controls and 28

[0.8%] in cases), Mixed (35 [0.3%] in controls and 18 [0.5%] in cases), and Chinese (11 [0.1%] in controls and 6 [0.2%] in cases).

Study participants were enrolled at 22 assessment centers in London (Barts, Croydon, and Hounslow), North East England (Middlesbrough and Newcastle), South East England (Oxford and Reading), North West England (Bury, Liverpool, and Manchester), South West England (Bristol), West (Stoke and Birmingham) and East (Nottingham) midlands of England, Yorkshire and the Humber (Leeds and Sheffield), Scotland (Edinburgh and Glasgow), and Wales (Swansea, Wrexham, and Cardiff). Study participants enrolled the most in West England (Bristol, 887 [8.4] in controls and 296 [8.4] in cases; Bury, 699 [6.6%] in controls and 236 [6.7%] in cases; Liverpool, 673 [6.4%] in controls and 226 [6.4%] in cases; Manchester, 339 [3.2%] in controls and 114 [3.2%] in cases; Stockport, 12 [0.1%] in controls and 5 [0.1%] in cases), followed by East England (Newcastle, 903 [8.6%] in controls and 300 [8.5%] in cases; Reading, 682 [6.5%] in controls and 229 [6.5%] in cases; Oxford, 378 [3.6%] in controls and 128 [3.6%] in cases; Middlesbrough, 353 [3.4%] in controls and 118 [3.3%] in cases), Midlands

(Nottingham, 704 [6.7%] in controls and 236 [6.7%] in cases; Stoke, 460 [4.4%] in controls and 154 [4.4%] in cases; Birmingham, 399 [3.8%] in controls and 135 [3.8%] in cases), Yorkshire and the Humber (Leeds, 928 [8.8%] in controls and 309 [8.8%] in cases; Sheffield, 572 [5.4%] in controls and 193 [5.5%] in cases), London (Hounslow, 472 [4.5%] in controls and 157 [4.5%] in cases; Croydon, 408 [3.9%] in controls and 136 [3.9%] in cases; Barts, 230 [2.2%] in controls and 76 [2.2%] in cases), Scotland (Glasgow, 474 [4.5%] in controls and 158 [4.5%] in cases; Edinburgh, 445 [4.2%] in controls and 148 [4.2%] in cases), and Wales (Cardiff, 453 [4.3%] in controls and 152 [4.3%] in cases; Swansea, 45 [0.4%] in controls and 15 [0.4%] in cases; Wrexham, 6 [0.1%] in controls and 2 [0.1%] in cases).

Participants whose Townsend deprivation index ranged from -6.26 to -3.65 were 2,788 (26.5%) in controls and 933 (26.5%) in cases, -3.65 to -2.15 were 2,733 (26.0%) in controls and 916 (26.0%) in cases, -2.15 to 0.515 were 2,450 (23.3%) in controls and 820 (23.3%) in cases, 0.515 to 11 were 2,551 (24.2%) in controls and 854 (24.2%) in cases.

Table 9. The number and proportion of control and colorectal cancer cases in a nested case-control study from the participants of the UK Biobank.

Characteristics and categories	Controls, n (%)	Colorectal cancer cases, n (%)
Number of participants	10,522	3,523
Age at enrollment, years		
36-40	24 (0.2)	8 (0.2)
41-45	272 (2.6)	93 (2.6)
46-50	575 (5.5)	193 (5.5)
51-55	1,237 (11.8)	412 (11.7)
56-60	2,007 (19.1)	673 (19.1)
61-65	3,361 (31.9)	1,123 (31.9)
66-70	3,046 (28.9)	1,021 (29.0)
Sex		
Men	6,052 (57.5)	2,024 (57.5)
Women	4,470 (42.5)	1,499 (42.5)
Ethnic background		
White	10,284 (97.7)	3,423 (97.2)
Mixed	35 (0.3)	18 (0.5)
Asian or Asian British	83 (0.8)	31 (0.9)
Black or Black British	76 (0.7)	28 (0.8)
Chinese	11 (0.1)	6 (0.2)
Other	33 (0.3)	17 (0.5)
Assessment center at which participant consented		
Barts	230 (2.2)	76 (2.2)
Birmingham	399 (3.8)	135 (3.8)
Bristol	887 (8.4)	296 (8.4)
Bury	699 (6.6)	236 (6.7)
Cardiff	453 (4.3)	152 (4.3)
Croydon	408 (3.9)	136 (3.9)
Edinburgh	445 (4.2)	148 (4.2)
Glasgow	474 (4.5)	158 (4.5)
Hounslow	472 (4.5)	157 (4.5)
Leeds	928 (8.8)	309 (8.8)
Liverpool	673 (6.4)	226 (6.4)
Manchester	339 (3.2)	114 (3.2)
Middlesbrough	353 (3.4)	118 (3.3)
Newcastle	903 (8.6)	300 (8.5)
Nottingham	704 (6.7)	236 (6.7)
Oxford	378 (3.6)	128 (3.6)
Reading	682 (6.5)	229 (6.5)
Sheffield	572 (5.4)	193 (5.5)
Stockport	12 (0.1)	5 (0.1)
Stoke	460 (4.4)	154 (4.4)
Swansea	45 (0.4)	15 (0.4)
Wrexham	6 (0.1)	2 (0.1)
Townsend deprivation index at recruitment		
[-6.26,-3.65]	2,788 (26.5)	933 (26.5)
(-3.65,-2.15]	2,733 (26.0)	916 (26.0)
(-2.15,0.515]	2,450 (23.3)	820 (23.3)
(0.515,11]	2,551 (24.2)	854 (24.2)

Table 10 shows the association between the contributors to energy balance, including obesity, physical activity, and energy intake, and the risk of colorectal cancer by subsites. General obesity is significantly associated with colon cancer (1.25 [1.13-1.39]), although significant associations between severe obesity and the cancer of the colon (1.19 [0.84-1.69]) and rectum (1.11 [0.67-1.83]) were not found. Abdominal obesity defined using WHR was associated with an increased risk for colon cancer (1.30 [1.17-1.45]) and rectal cancer (1.22 [1.05-1.42]) with statistical significance. Participants who did sufficient moderate physical activity had a decreased risk of colon cancer (0.88 [0.78-0.99]). Sufficient vigorous physical activity was associated with a decreased risk for colon and rectum cancer (0.93 [0.79-1.09] and 0.98 [0.81-1.20], respectively), but these associations did not show statistical significance. Participants who reported excess energy intake had an increased risk for colon (1.18 [0.81-1.73]) and rectal cancer (1.11 [0.63-1.96)), but these associations did not reach statistical significance.

Table 10. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) of obesity, physical activity, and energy intake for the risk of colorectal cancer by subsites.

	Colon cancer			Rectal cancer		
Lifestyle factors	Controls, n(%)	Cases, n(%)	OR (95% CIs)	Controls, n(%)	Cases, n(%)	OR (95% CIs)
Obesity, BMI						
<30 kg/m^2	5,279 (75.3)	1,664 (71.1)	1.00 (reference)	2,730 (76.1)	899 (75.0)	1.00 (reference)
≥30 kg/m^2	1,730 (24.7)	675 (28.9)	**1.25 (1.13-1.39)**	858 (23.9)	300 (25.0)	1.07 (0.91-1.24)
Severe obesity, BMI						
<40 kg/m^2	6,891 (98.3)	2,293 (98.0)	1.00 (reference)	3,531 (98.4)	1,178 (98.2)	1.00 (reference)
≥40 kg/m^2	118 (1.7)	46 (2.0)	1.19 (0.84-1.69)	57 (1.6)	21 (1.8)	1.11 (0.67-1.83)
Abdominal obesity, WHR						
Men, ≤0.9; women, ≤0.85	3,106 (44.3)	920 (39.2)	1.00 (reference)	1,453 (40.4)	442 (36.8)	1.00 (reference)
Men, >0.9; women,>0.85	3,908 (55.7)	1,427 (60.8)	**1.30 (1.17-1.45)**	2,141 (59.6)	759 (63.2)	**1.22 (1.05-1.42)**
Moderate physical activity*						
Not sufficient	2,502 (48.1)	893 (52.5)	1.00 (reference)	1,287 (47.9)	406 (45.4)	1.00 (reference)
Sufficient	2,701 (51.9)	809 (47.5)	**0.88 (0.78-0.99)**	1,402 (52.1)	489 (54.6)	1.11 (0.94-1.32)
Vigorous physical activity*						
Not sufficient	1,729 (46.9)	561 (49.9)	1.00 (reference)	863 (45.3)	285 (43.7)	1.00 (reference)
Sufficient	1,958 (53.1)	563 (50.1)	0.93 (0.79-1.09)	1,043 (54.7)	367 (56.3)	0.98 (0.81-1.20)
Moderate or vigorous physical activity*						
Not sufficient	892 (20.9)	323 (24.1)	1.00 (reference)	445 (20.0)	138 (18.3)	1.00 (reference)
Sufficient	3,386 (79.1)	1,015 (75.9)	0.93 (0.78-1.09)	1,776 (80.0)	618 (81.7)	1.10 (0.86-1.39)
Daily energy intake, kcal						
Men, <2000; women,<2500	401 (47.6)	120 (44.8)	1.00 (reference)	176 (42.2)	54 (39.4)	1.00 (reference)
Men, <2000; women, ≥2500	442 (52.4)	148 (55.2)	1.18 (0.81-1.73)	241 (57.8)	83 (60.6)	1.11 (0.63-1.96)

ORs denotes odd ratios, 95% CIs 95% confidence intervals, BMI body mass index, and WHR waist-hip ratio.

Bold font indicates the statistical significance with a p-value of less than 0.05.

* Participants who performed over 150 min of moderate physical activity or 75 min of vigorous physical activity throughout the week were classified as people who achieved the physical activity necessary for experiencing general health benefits.

4.2. Citric acid cycle polymorphisms involved in the risk for colon and rectal cancer development

Table 11 shows the association between citric acid cycle-SNPs and colorectal cancer by subsites. *SUCLG2*-rs35494829 were associated with a decreased risk for colon cancer in the dominant model (ORs [95% CIs]; 0.82 [0.74–0.92]) and the additive model (0.82 [0.74–0.92]; p=0.000981). The significance of the association between *SUCLG2*-rs35494829 and colon cancer remained after correcting multiple comparisons using FDR (p=0.0206).

Figure 5 presents the regional association plot for 21 included SNPs at each gene locus. The color scheme indicates linkage disequilibrium between the top-ranked SNP and other SNPs in the region using r^2 values calculated from the 1000 Genomes Project. The y-axis shows −log10 (P) values computed from 3,523 colorectal cancer cases and 10,522 controls. The recombination rate (right y-axis) is shown in blue based on the 2014 European HapMap data.

Table 11. Odds ratios (ORs) and 95% confidence intervals (95% CIs) of the association of SNPs in genes of the citric acid cycle with the risk of colon and rectal cancer.

	Colon cancer			Rectal cancer		
Gene-SNP	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)
SDHC-rs16832884						
CC	6183 (88.0)	2061 (87.6)	1.00 (reference)	3190 (88.7)	1059 (88.0)	1.00 (reference)
CT	816 (11.6)	285 (12.1)	1.04 (0.90–1.20)	393 (10.9)	140 (11.6)	1.08 (0.87–1.32)
TT	27 (0.4)	6 (0.3)	0.64 (0.26–1.55)	14 (0.4)	4 (0.3)	0.85 (0.28–2.59)
CT + TT			1.03 (0.89–1.19)			1.07 (0.87–1.31)
Per T allele			1.01 (0.88–1.16)			1.06 (0.87–1.29)
SDHC-rs17395595						
AA	5062 (72.4)	1680 (71.6)	1.00 (reference)	2616 (73.0)	895 (74.5)	1.00 (reference)
AG	1785 (25.5)	613 (26.1)	1.04 (0.94–1.16)	883 (24.6)	280 (23.3)	0.93 (0.80–1.08)
GG	142 (2.0)	52 (2.2)	1.11 (0.81–1.54)	84 (2.3)	26 (2.2)	0.89 (0.57–1.39)
AG + GG			1.05 (0.94–1.16)			0.93 (0.80–1.07)
Per G allele			1.05 (0.95–1.15)			0.93 (0.82–1.07)
MDH1-rs2278718						
GG	3991 (56.9)	1350 (57.4)	1.00 (reference)	2003 (55.7)	688 (57.3)	1.00 (reference)
GA	2580 (36.8)	857 (36.5)	0.98 (0.89–1.09)	1365 (38.0)	435 (36.2)	0.93 (0.81–1.07)
AA	444 (6.3)	143 (6.1)	0.95 (0.78–1.16)	228 (6.3)	78 (6.5)	1.00 (0.77–1.32)
GA + AA			0.98 (0.89–1.08)			0.94 (0.83–1.07)
Per A allele			0.98 (0.91–1.06)			0.97 (0.87–1.07)
IDH1-rs34218846						
GG	6252 (89.0)	2100 (89.3)	1.00 (reference)	3212 (89.4)	1080 (89.9)	1.00 (reference)
GA	750 (10.7)	245 (10.4)	0.98 (0.84–1.14)	368 (10.2)	117 (9.7)	0.95 (0.77–1.18)
AA	19 (0.3)	6 (0.3)	0.93 (0.37–2.33)	13 (0.4)	5 (0.4)	1.07 (0.38–3.03)
GA + AA			0.97 (0.84–1.13)			0.96 (0.77–1.18)
Per A allele			0.97 (0.84–1.13)			0.96 (0.79–1.18)
SUCLG2-rs902320						
GG	3753 (53.4)	1280 (54.5)	1.00 (reference)	1921 (53.5)	650 (54.0)	1.00 (reference)
GA	2730 (38.9)	907 (38.6)	0.97 (0.88–1.08)	1415 (39.4)	476 (39.6)	0.99 (0.87–1.14)
AA	541 (7.7)	162 (6.9)	0.87 (0.72–1.05)	256 (7.1)	77 (6.4)	0.89 (0.69–1.17)
GA + AA			0.96 (0.87–1.05)			0.98 (0.86–1.11)
Per A allele			0.95 (0.88–1.03)			0.97 (0.87–1.07)

ORs denotes odd ratios, 95% CIs 95% confidence intervals, SNP denotes single nucleotide polymorphism.
Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 11. Continued.

Gene-SNP	Colon cancer			Rectal cancer		
	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)
SUCLG2-rs902321						
TT	2605 (37.2)	870 (37.1)	1.00 (reference)	1315 (36.6)	439 (36.7)	1.00 (reference)
TG	3282 (46.9)	1118 (47.7)	1.02 (0.92–1.13)	1701 (47.4)	586 (49.0)	1.03 (0.89–1.19)
GG	1114 (15.9)	358 (15.3)	0.96 (0.84–1.11)	572 (15.9)	172 (14.4)	0.90 (0.74–1.10)
TG + GG			1.00 (0.91–1.10)			1.00 (0.87–1.14)
Per G allele			0.99 (0.92–1.06)			0.97 (0.88–1.06)
SUCLG2-rs35494829						
CC	5516 (78.7)	1919 (81.9)	1.00 (reference)	2812 (78.7)	945 (78.8)	1.00 (reference)
CT	1402 (20.0)	404 (17.3)	**0.83 (0.73–0.94)**	715 (20.0)	241 (20.1)	1.00 (0.85–1.18)
TT	87 (1.2)	19 (0.8)	0.64 (0.39–1.05)	47 (1.3)	14 (1.2)	0.89 (0.49–1.63)
CT + TT			**0.82 (0.72–0.92)**			1.00 (0.85–1.17)
Per T allele			**0.82 (0.74–0.92)**			0.99 (0.86–1.15)
SUCLG2-rs2363712						
TT	3184 (45.4)	1087 (46.3)	1.00 (reference)	1643 (45.8)	560 (46.6)	1.00 (reference)
TC	3048 (43.5)	1018 (43.4)	0.98 (0.89–1.08)	1561 (43.5)	531 (44.1)	1.00 (0.87–1.14)
CC	777 (11.1)	242 (10.3)	0.91 (0.77–1.07)	386 (10.8)	112 (9.3)	0.86 (0.68–1.08)
TC + CC			0.97 (0.88–1.06)			0.97 (0.85–1.10)
Per C allele			0.96 (0.90–1.03)			0.95 (0.86–1.05)
SDHA-rs6962						
GG	5297 (75.5)	1787 (76.0)	1.00 (reference)	2726 (75.9)	896 (74.5)	1.00 (reference)
GT	1605 (22.9)	529 (22.5)	0.98 (0.87–1.09)	808 (22.5)	294 (24.4)	1.10 (0.94–1.28)
TT	110 (1.6)	34 (1.4)	0.92 (0.62–1.35)	57 (1.6)	13 (1.1)	0.68 (0.37–1.26)
GT + TT			0.97 (0.87–1.09)			1.08 (0.92–1.25)
Per T allele			0.97 (0.88–1.07)			1.04 (0.90–1.19)
SDHA-rs34511054						
GG	6202 (88.5)	2063 (88.0)	1.00 (reference)	3176 (88.6)	1065 (88.7)	1.00 (reference)
GA	776 (11.1)	275 (11.7)	1.07 (0.92–1.24)	399 (11.1)	130 (10.8)	0.97 (0.79–1.20)
AA	31 (0.4)	7 (0.3)	0.67 (0.30–1.53)	9 (0.3)	6 (0.5)	1.99 (0.71–5.60)
GA + AA			1.05 (0.91–1.22)			1.00 (0.81–1.22)
Per A allele			1.03 (0.90–1.19)			1.02 (0.84–1.24)

ORs denotes odd ratios, 95% CIs 95% confidence intervals, SNP denotes single nucleotide polymorphism.
Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 11. Continued.

Gene-SNP	Colon cancer			Rectal cancer		
	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)
ACO1-rs7042042						
GG	2898 (41.3)	946 (40.2)	1.00 (reference)	1501 (41.8)	515 (42.8)	1.00 (reference)
GA	3241 (46.2)	1110 (47.2)	1.05 (0.95–1.16)	1619 (45.1)	544 (45.2)	0.98 (0.85–1.13)
AA	880 (12.5)	295 (12.5)	1.02 (0.88–1.19)	467 (13.0)	144 (12.0)	0.90 (0.73–1.11)
GA + AA			1.04 (0.95–1.15)			0.96 (0.84–1.10)
Per A allele			1.02 (0.95–1.10)			0.96 (0.87–1.05)
ACO1-rs10970986						
AA	3561 (50.8)	1174 (50.0)	1.00 (reference)	1776 (49.5)	586 (48.7)	1.00 (reference)
AG	2850 (40.6)	992 (42.2)	1.06 (0.96–1.17)	1510 (42.0)	501 (41.6)	1.01 (0.88–1.16)
GG	603 (8.6)	183 (7.8)	0.93 (0.78–1.11)	305 (8.5)	116 (9.6)	1.15 (0.91–1.45)
AG + GG			1.04 (0.94–1.14)			1.03 (0.91–1.18)
Per G allele			1.00 (0.93–1.08)			1.05 (0.95–1.16)
OGDHL-rs11101224						
AA	4690 (66.8)	1557 (66.2)	1.00 (reference)	2438 (67.9)	825 (68.5)	1.00 (reference)
AG	2121 (30.2)	717 (30.5)	1.02 (0.92–1.13)	1046 (29.1)	351 (29.2)	0.99 (0.86–1.15)
GG	209 (3.0)	77 (3.3)	1.12 (0.85–1.46)	109 (3.0)	28 (2.3)	0.76 (0.49–1.16)
AG + GG			1.03 (0.93–1.13)			0.97 (0.84–1.12)
Per G allele			1.03 (0.94–1.12)			0.95 (0.84–1.08)
DLAT-rs10891314						
GG	2759 (39.6)	996 (42.7)	1.00 (reference)	1442 (40.5)	486 (40.8)	1.00 (reference)
GA	3231 (46.4)	1073 (46.0)	0.92 (0.83–1.02)	1672 (46.9)	557 (46.8)	0.99 (0.86–1.14)
AA	975 (14.0)	266 (11.4)	**0.75 (0.65–0.88)**	450 (12.6)	148 (12.4)	0.98 (0.79–1.22)
GA + AA			**0.88 (0.80–0.97)**			0.99 (0.86–1.13)
Per A allele			**0.88 (0.82–0.95)**			0.99 (0.90–1.09)
PCK2-rs55733026						
AA	6050 (86.2)	2048 (87.1)	1.00 (reference)	3060 (85.1)	1042 (86.8)	1.00 (reference)
AG	937 (13.3)	296 (12.6)	0.93 (0.81–1.07)	516 (14.4)	147 (12.2)	0.83 (0.68–1.01)
GG	35 (0.5)	8 (0.3)	0.68 (0.32–1.47)	18 (0.5)	12 (1.0)	2.03 (0.95–4.33)
AG + GG			0.92 (0.80–1.06)			0.87 (0.72–1.05)
Per G allele			0.92 (0.80–1.05)			0.92 (0.77–1.10)

ORs denotes odd ratios, 95% CIs 95% confidence intervals, SNP denotes single nucleotide polymorphism.
Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 11. Continued.

Gene-SNP	Colon cancer			Rectal cancer		
	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)
PCK2-rs1951634						
CC	3918 (55.9)	1314 (56.0)	1.00 (reference)	1993 (55.6)	669 (55.7)	1.00 (reference)
CT	2652 (37.8)	881 (37.5)	0.99 (0.89–1.09)	1351 (37.7)	460 (38.3)	1.02 (0.89–1.17)
TT	444 (6.3)	152 (6.5)	1.01 (0.84–1.23)	243 (6.8)	72 (6.0)	0.88 (0.67–1.17)
CT + TT			0.99 (0.90–1.09)			1.00 (0.87–1.14)
Per T allele			1.00 (0.92–1.08)			0.98 (0.88–1.09)
PCK2-rs35618680						
GG	5798 (82.7)	1953 (83.3)	1.00 (reference)	2976 (83.0)	998 (83.2)	1.00 (reference)
GA	1156 (16.5)	370 (15.8)	0.95 (0.84–1.08)	580 (16.2)	194 (16.2)	0.99 (0.83–1.19)
AA	56 (0.8)	21 (0.9)	1.10 (0.67–1.83)	31 (0.9)	8 (0.7)	0.76 (0.35–1.66)
GA + AA			0.96 (0.85–1.09)			0.98 (0.82–1.17)
Per A allele			0.97 (0.86–1.09)			0.97 (0.83–1.14)
IDH3A-rs11555541						
AA	1823 (26.0)	600 (25.5)	1.00 (reference)	903 (25.2)	309 (25.7)	1.00 (reference)
AC	3447 (49.1)	1178 (50.1)	1.04 (0.93–1.16)	1814 (50.5)	595 (49.4)	0.96 (0.82–1.12)
CC	1749 (24.9)	575 (24.4)	1.00 (0.87–1.14)	872 (24.3)	300 (24.9)	1.00 (0.84–1.21)
AC + CC			1.03 (0.92–1.14)			0.97 (0.84–1.13)
Per C allele			1.00 (0.94–1.07)			1.00 (0.91–1.10)
IDH3A-rs17674205						
TT	5788 (83.1)	1957 (84.0)	1.00 (reference)	2949 (82.7)	1008 (84.1)	1.00 (reference)
TC	1127 (16.2)	357 (15.3)	0.94 (0.83–1.07)	588 (16.5)	180 (15.0)	0.90 (0.75–1.08)
CC	51 (0.7)	16 (0.7)	0.96 (0.54–1.70)	28 (0.8)	10 (0.8)	1.09 (0.53–2.26)
TC + CC			0.94 (0.83–1.07)			0.91 (0.76–1.08)
Per C allele			0.95 (0.84–1.07)			0.92 (0.78–1.09)
ACLY-rs8065502						
AA	5890 (83.9)	1959 (83.3)	1.00 (reference)	3009 (83.8)	1017 (84.5)	1.00 (reference)
AG	1060 (15.1)	377 (16.0)	1.07 (0.94–1.21)	561 (15.6)	180 (15.0)	0.95 (0.79–1.14)
GG	67 (1.0)	16 (0.7)	0.72 (0.42–1.25)	21 (0.6)	7 (0.6)	0.99 (0.42–2.32)
AG + GG			1.04 (0.92–1.18)			0.95 (0.79–1.14)
Per G allele			1.02 (0.91–1.15)			0.95 (0.80–1.13)

ORs denotes odd ratios, 95% CIs 95% confidence intervals, SNP denotes single nucleotide polymorphism.
Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 11. Continued.

Gene-SNP	Colon cancer			Rectal cancer		
	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)
ACLY-rs2304497						
GG	5414 (77.2)	1790 (76.2)	1.00 (reference)	2721 (75.7)	923 (76.7)	1.00 (reference)
GA	1482 (21.1)	524 (22.3)	1.07 (0.95–1.20)	813 (22.6)	258 (21.4)	0.94 (0.80–1.10)
AA	121 (1.7)	35 (1.5)	0.88 (0.60–1.28)	60 (1.7)	22 (1.8)	1.09 (0.66–1.78)
GA + AA			1.05 (0.94–1.18)			0.95 (0.82–1.11)
Per A allele			1.03 (0.93–1.14)			0.97 (0.84–1.11)

ORs denotes odd ratios, 95% CIs 95% confidence intervals, SNP denotes single nucleotide polymorphism.
Bold font indicates the statistical significance with a p-value of less than 0.05.

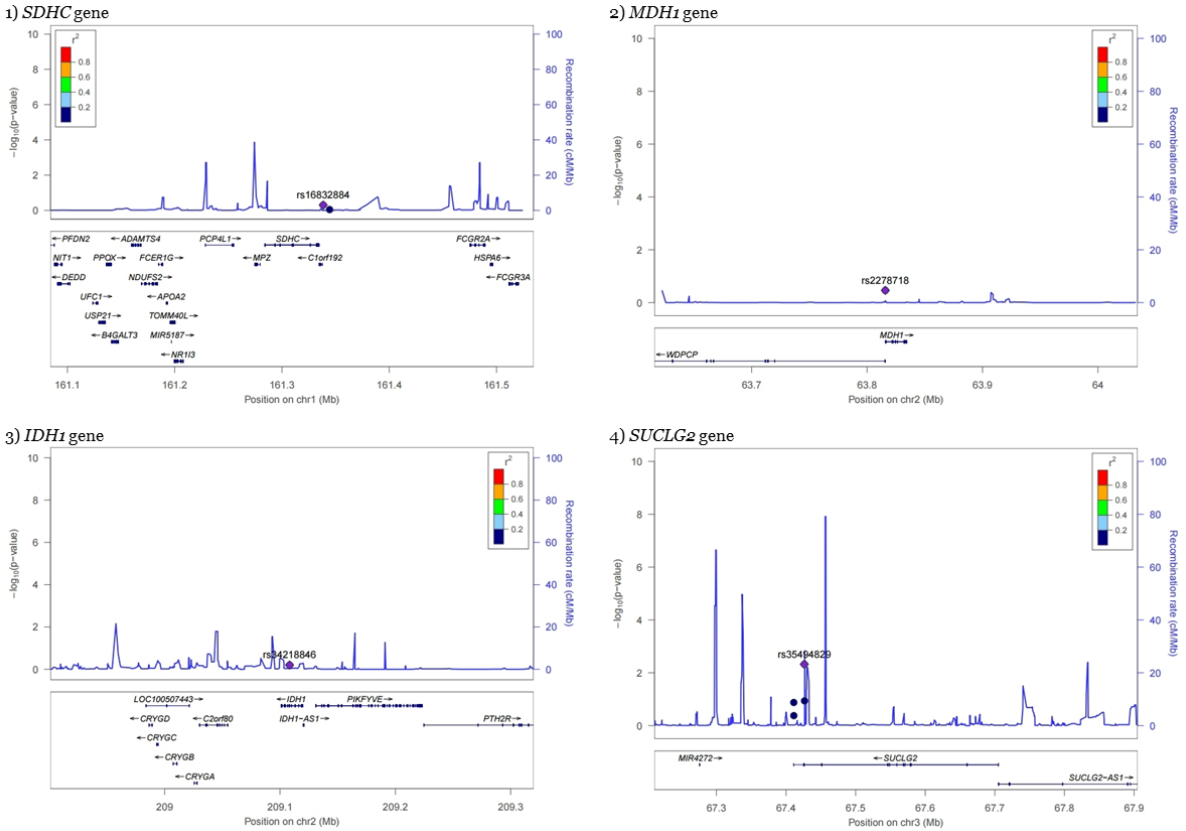


Figure 5. Regional association plot for 21 SNPs at each gene locus

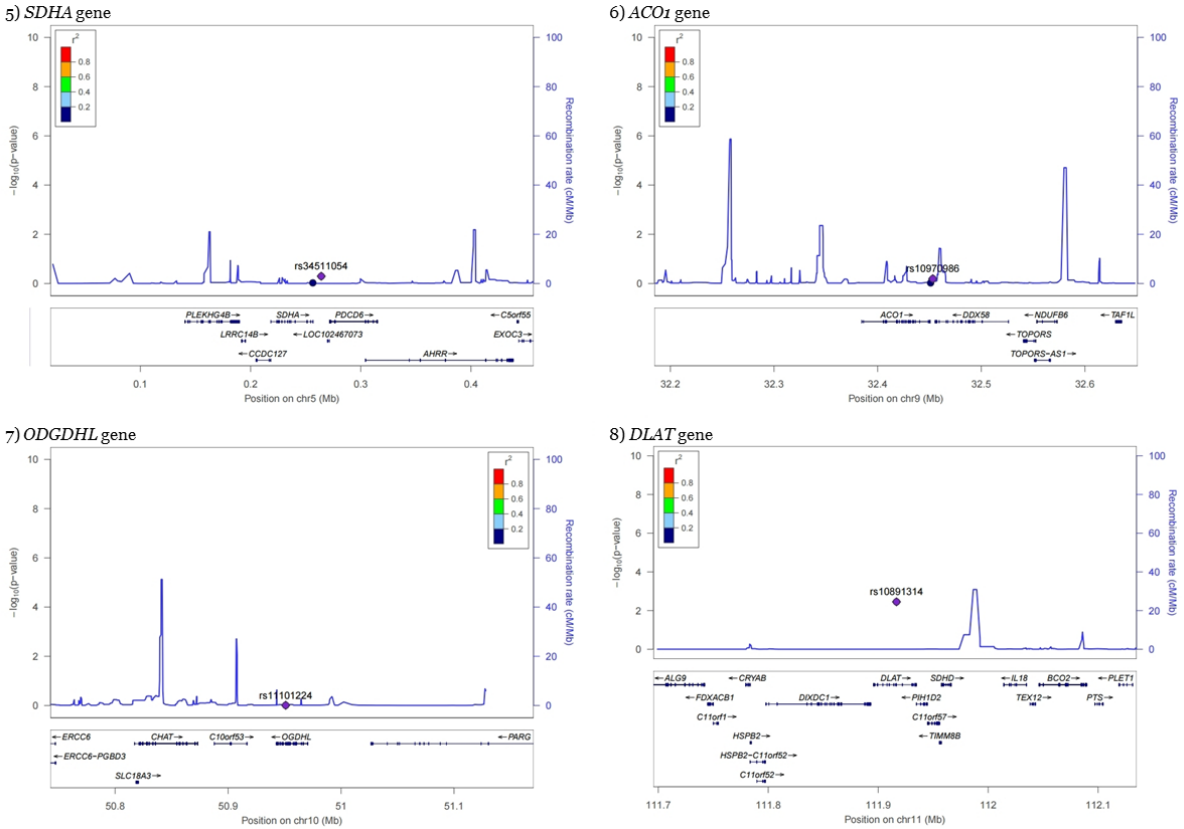
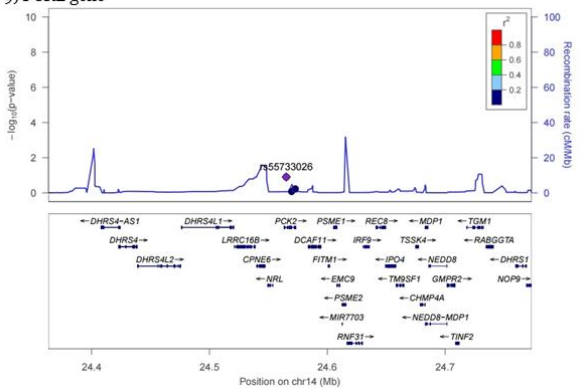
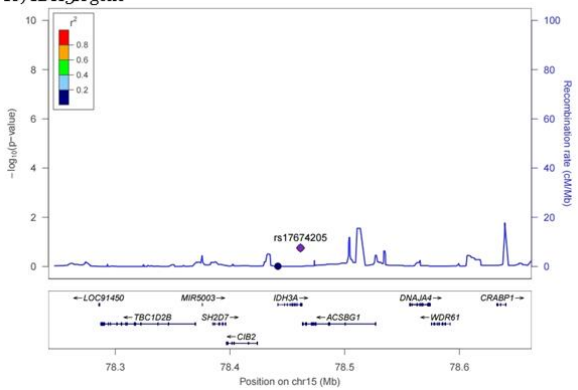


Figure 5. Continued

9) *PCK2* gene



10) *IDH3A* gene



11) *ACLY* gene

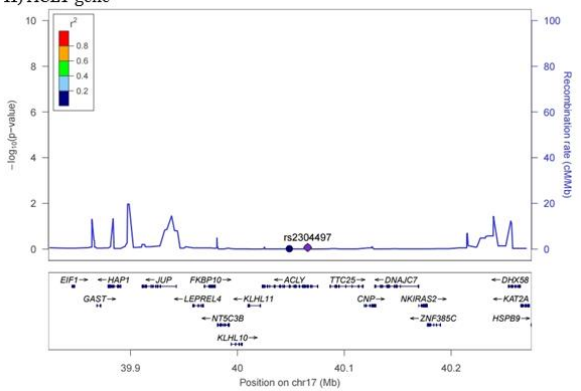


Figure 5. Continued

4.3. Interaction of the citric acid cycle polymorphisms with obesity, physical activity, and energy intake on the risk of colorectal cancer development

The interaction between SNPs in the gene encoding the component of the citric acid cycle and the contributors of energy balance in the development of colorectal cancer were investigated. Odds ratios and corresponding 95% confidence intervals of environmental factors have been presented by minor allele noncarrier and carrier only if p-values of interaction were observed under 0.05.

Table 12 shows the OR and 95% CIs for the contributors of energy balance on the risk of colon cancer by minor allele noncarriers or carriers of the citric acid cycle SNPs showing the interaction of p-value under 0.05. The significant interactions on colon cancer were found as follows: obesity and *SDHC*-rs17395595 ($p_{interaction}$ = 0.0023), severe obesity and *MDH1*-rs2278718 ($p_{interaction}$ = 0.0229), severe obesity and *SUCLG2*-rs902320 ($p_{interaction}$ = 0.0437), severe obesity and *SUCLG2*-rs902321 ($p_{interaction}$ = 0.0071), abdominal obesity and *PCK2*-rs55733026 ($p_{interaction}$ = 0.0376), vigorous physical activity and *ACLY*-rs2304497 ($p_{interaction}$ =

0.0450). Obesity was associated with an increased risk for colon cancer among minor allele noncarriers of *SDHC*-rs17395595 (1.42 [1.24-1.63]). Severe obesity was associated with an increased risk for colon cancer among minor allele carriers of *SUCLG2*-rs902321 (1.74 [1.07-2.82]). The significance of the interaction between obesity and *SDHC*-rs17395595 for colon cancer remained after correcting multiple comparisons using FDR ($p_{interaction}$=0.047344).

Table 12. Odds ratios (OR) and 95% confidence intervals (CIs) for the contributors of energy balance on the risk of colon cancer by minor allele noncarriers or carriers of the citric acid cycle SNPs showing an interaction p-value under 0.05.

	Noncarriers			Carriers			
Environmental Variable	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	$P_{interaction}$
			SDHC-rs17395595, G < A				
Obesity, BMI							0.0023
< 30 kg/m^2	3833 (75.9)	1163 (69.8)	1.00 (reference)	1416 (73.7)	495 (74.5)	1.00 (reference)	
≥ 30 kg/m^2	1216 (24.1)	504 (30.2)	**1.42 (1.24–1.63)**	506 (26.3)	169 (25.5)	0.92 (0.69–1.23)	
			MDH1-rs2278718, C < A				
Severe obesity, BMI							0.0229
< 40 kg/m^2	3906 (98.1)	1317 (98.4)	1.00 (reference)	2973 (98.6)	973 (97.5)	1.00 (reference)	
≥ 40 kg/m^2	76 (1.9)	21 (1.6)	1.18 (0.67–2.07)	42 (1.4)	25 (2.5)	1.47 (0.76–2.84)	
			SUCLG2-rs902320, T < C				
Severe obesity, BMI							0.0437
< 40 kg/m^2	3672 (98.0)	1250 (98.3)	1.00 (reference)	3216 (98.7)	1039 (97.7)	1.00 (reference)	
≥ 40 kg/m^2	76 (2.0)	22 (1.7)	0.89 (0.50–1.59)	42 (1.3)	24 (2.3)	1.95 (1.00–3.81)	
			SUCLG2-rs902321, G < A				
Severe obesity, BMI							0.0071
< 40 kg/m^2	2544 (97.8)	851 (98.6)	1.00 (reference)	4321 (98.6)	1435 (97.7)	1.00 (reference)	
≥ 40 kg/m^2	58 (2.2)	12 (1.4)	0.82 (0.39–1.69)	60 (1.4)	34 (2.3)	**1.74 (1.07–2.82)**	
			PCK2-rs55733026, G < A				
Abdominal obesity, WHR							0.0376
Men, ≤ 0.9; women, ≤ 0.85	340 (47.0)	99 (43.4)	1.00 (reference)	61 (50.8)	21 (52.5)	1.00 (reference)	
Men, > 0.9; women, > 0.85	383 (53.0)	129 (56.6)	1.22 (0.79–1.90)	59 (49.2)	19 (47.5)	0.86 (0.45–1.45)	
			ACLY-rs2304497, G < T				
Vigorous physical activity*							0.045
Not sufficient	1304 (46.0)	434 (50.3)	1.00 (reference)	422 (50.0)	127 (48.7)	1.00 (reference)	
Sufficient	1533 (54.0)	428 (49.7)	0.84 (0.68-1.02)	422 (50.0)	134 (51.3)	0.69 (0.36-1.33)	

ORs denotes odd ratios, 95% CIs 95% confidence intervals, BMI denotes body mass index, and WHR denotes waist-hip ratio.

Bold font indicates the statistical significance with a p-value of less than 0.05.

* Participants who performed over 75 min of vigorous physical activity throughout the week were classified as people who achieved the physical activity necessary for experiencing general health benefits.

Odds ratios and corresponding 95% confidence intervals for energy balance-related environmental factors for rectal cancer by minor allele noncarrier and carrier are shown in Table 13. The significant interactions on rectal cancer were found as follows: obesity and *MDH1*- rs2278718 ($p_{interaction}$ = 0.0450), severe obesity and *SUCLG2*-rs902321 ($p_{interaction}$ = 0.0468), severe obesity and *SUCLG2*-rs35494829 ($p_{interaction}$ = 0.0457), abdominal obesity and *SUCLG2*-rs35494829 ($p_{interaction}$ = 0.0159), abdominal obesity and *OGDHL*-rs11101224 ($p_{interaction}$ = 0.0193). Obesity was associated with an increased risk for rectal cancer among minor allele carriers of *MDH1*-rs2278718 (1.39 [1.04–1.87]). Abdominal obesity was associated with an increased risk for rectal cancer among minor allele noncarriers of *SUCLG2*-rs35494829 (1.35 [1.12–1.63]).

Table 13. Odds ratios (OR) and 95% confidence intervals (CIs) for the contributors of energy balance on the risk of rectal cancer by minor allele noncarriers and carriers of the citric acid cycle SNPs showing an interaction p-value under 0.05

Environmental variable	Noncarriers			Carriers			$P_{interaction}$
	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	Controls, *n* (%)	Cases, *n* (%)	OR (95% CIs)	
			MDH1-rs2278718, C < A				
Obesity, BMI							0.045
< 30 kg/m^2	1503 (75.3)	528 (76.7)	1.00 (reference)	1227 (77.1)	370 (72.8)	1.00 (reference)	
≥ 30 kg/m^2	493 (24.7)	160 (23.3)	0.91 (0.72–1.15)	364 (22.9)	138 (27.2)	**1.39 (1.04–1.87)**	
			SUCLG2-rs902321, G < A				
Severe obesity, BMI							0.0468
< 40 kg/m^2	1283 (97.7)	432 (98.6)	1.00 (reference)	2240 (98.9)	739 (98.0)	1.00 (reference)	
≥ 40 kg/m^2	30 (2.3)	6 (1.4)	0.40 (0.13–1.24)	26 (1.1)	15 (2.0)	1.38 (0.67–2.84)	
			SUCLG2-rs35494829, C < T				
Severe obesity, BMI							0.0457
< 40 kg/m^2	2766 (98.6)	921 (98.0)	1.00 (reference)	742 (97.8)	253 (99.2)	1.00 (reference)	
≥ 40 kg/m^2	40 (1.4)	19 (2.0)	1.51 (0.83–2.75)	17 (2.2)	2 (0.8)	0.39 (0.04–3.78)	
Abdominal obesity, WHR							0.0159
Men, ≤ 0.9; women, ≤ 0.85	1152 (41.0)	333 (35.4)	1.00 (reference)	290 (38.1)	107 (42.0)	1.00 (reference)	
Men, > 0.9; women, > 0.85	1658 (59.0)	609 (64.6)	**1.35 (1.12–1.63)**	471 (61.9)	148 (58.0)	1.11 (0.64–1.94)	
			OGDHL-rs11101224, A < G				
Abdominal obesity, WHR							0.0193
Men, ≤ 0.9; women, ≤ 0.85	1003 (41.2)	288 (35.0)	1.00 (reference)	448 (38.8)	154 (40.7)	1.00 (reference)	
Men, > 0.9; women, > 0.85	1432 (58.8)	535 (65.0)	**1.37 (1.12–1.68)**	707 (61.2)	224 (59.3)	1.08 (0.75–1.55)	

ORs denotes odd ratios, 95% CIs 95% confidence intervals, BMI denotes body mass index, and WHR denotes waist-hip ratio.
Bold font indicates the statistical significance with a p-value of less than 0.05.

4.4. Pairwise SNP-SNP interactions of SNPs within the Citric acid cycle on the risk of colorectal cancer

Table 14 presents the results of the SNP-SNP interactions for colon cancer, showing that the 95% CIs of AP do not contain zero. The AP for colon cancer are shown as follows: *SDHC*-rs17395595 and *IDH3A*-rs11555541 (-0.348 [-0.628–0.068]), *MDH1*-rs2278718 and *SUCLG2*-rs902321 (-0.301 [-0.525–0.077]), *IDH1*-rs34218846 and *IDH3A*-rs11555541 (-0.507 [-0.978–0.036]), *SUCLG2*-rs902320 and *IDH3A*-rs17674205 (-0.570 [-0.966–0.174]), *SUCLG2*-rs902321 and *IDH3A*-rs11555541 (-0.258 [-0.500–0.016]), *SUCLG2*-rs902321 and *IDH3A*-rs17674205 (-0.491 [-0.862–0.121]), *SUCLG2*-rs35494829 and *IDH3A*-rs17674205 (-0.358 [-0.716–0.001]), *SUCLG2*-rs2363712 and *IDH3A*-rs11555541 (-0.282 [-0.508–0.055]), *SUCLG2*-rs2363712 and *IDH3A*-rs17674205 (-0.496 [-0.866–0.126]), *DLAT*-rs10891314 and *IDH3A*-rs11555541 (-0.288 [-0.530–0.046]).

Table 15 shows the results of the SNP-SNP interactions for rectal cancer, showing that the 95% CIs of AP did not contain zero. APs between *SDHC*-rs17395595 and *IDH3A*-rs11555541 (-0.341 [-0.672–0.010]), *SUCLG2*-rs902320

and *SDHA*-rs6962 (-0.390 [-0.774–0.006]), *SUCLG2*-rs902321 and *ACO1*-rs7042042 (-0.431 [-0.784–0.078]), *SUCLG2*-rs2363712 and *ACO1*-rs7042042 (-0.368 [-0.681–0.054]), *SDHA*-rs34511054, and *ACLY*-rs2304497 (-0.704 [-1.362–0.047]) were found to be negative, indicating that the interactions are less than additivity.

Table 14. Odds ratios and 95% confidence intervals for the combined effect of the SNPs within the citric acid cycle pathway on the risk for colon cancer, showing the 95% CIs of AP not containing zero.

Gene-SNP	Gene-SNP	Controls, n(%)	Cases, n(%)	ORs (95% CIs)	AP (95% CIs)
SDHC-rs17395595	*IDH3A*-rs11555541				-0.348 (-0.628--0.068)
AA	AA	1,357 (19.4)	414 (17.7)	1.00 (reference)	
AG+GG	AA	455 (6.5)	184 (7.8)	**1.33 (1.09-1.63)**	
AA	AC+CC	3,698 (53.0)	1,266 (54.0)	1.12 (0.99-1.27)	
AG+GG	AC+CC	1,471 (21.1)	481 (20.5)	1.08 (0.93-1.25)	
MDH1-rs2278718	*SUCLG2*-rs902321				-0.301 (-0.525--0.077)
GG	TT	1,484 (21.2)	463 (19.8)	1.00 (reference)	
GA+AA	TT	1,117 (16.0)	407 (17.4)	1.17 (1.00-1.37)	
GG	TG+GG	2,492 (35.7)	881 (37.6)	1.13 (1.00-1.29)	
GA+AA	TG+GG	1,896 (27.1)	592 (25.3)	1.00 (0.87-1.15)	
IDH1-rs34218846	*IDH3A*-rs11555541				-0.507 (-0.978--0.036)
GG	AA	1,638 (23.4)	522 (22.2)	1.00 (reference)	
GA+AA	AA	183 (2.6)	78 (3.3)	1.33 (1.00-1.76)	
GG	AC+CC	4,606 (65.7)	1,578 (67.1)	1.07 (0.96-1.20)	
GA+AA	AC+CC	586 (8.4)	173 (7.4)	0.93 (0.76-1.13)	
SUCLG2-rs902320	*IDH3A*-rs17674205				-0.570 (-0.966--0.174)
GG	TT	3,140 (45.1)	1,044 (44.9)	1.00 (reference)	
GA+AA	TT	2,645 (38.0)	909 (39.1)	1.03 (0.93-1.15)	
GG	TC+CC	579 (8.3)	222 (9.5)	1.17 (0.98-1.39)	
GA+AA	TC+CC	599 (8.6)	151 (6.5)	**0.76 (0.63-0.93)**	

AP denotes attributable proportion due to interaction, ORs odd ratios, 95% CIs 95% confidence intervals, SNP single nucleotide polymorphism.

Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 14. Continued.

Gene-SNP	Gene-SNP	Controls, n(%)	Cases, n(%)	ORs (95% CIs)	AP (95% CIs)
SUCLG2-rs902321	*IDH3A*-rs11555541				-0.258 (-0.500--0.016)
TG+GG	AA	1,169 (16.7)	359 (15.3)	1.00 (reference)	
TT	AA	648 (9.3)	240 (10.2)	1.20 (0.99–1.44)	
TG+GG	AC+CC	3,222 (46.1)	1,117 (47.6)	1.12 (0.98–1.29)	
TT	AC+CC	1,954 (27.9)	630 (26.9)	1.05 (0.91–1.22)	
SUCLG2-rs902321	*IDH3A*-rs17674205				-0.491 (-0.862--0.121)
TT	TT	2,174 (31.3)	703 (30.3)	1.00 (reference)	
TG+GG	TT	3,590 (51.7)	1,250 (53.8)	1.07 (0.96–1.19)	
TT	TC+CC	407 (5.9)	158 (6.8)	1.21 (0.98–1.48)	
TG+GG	TC+CC	769 (11.1)	212 (9.1)	0.86 (0.72–1.02)	
SUCLG2-rs35494829	*IDH3A*-rs17674205				-0.358 (-0.716--0.001)
CT+TT	TT	1,254 (18.1)	342 (14.7)	1.00 (reference)	
CC	TT	4,515 (65.0)	1,606 (69.2)	**1.31 (1.14–1.49)**	
CT+TT	TC+CC	223 (3.2)	76 (3.3)	1.26 (0.94–1.68)	
CC	TC+CC	952 (13.7)	296 (12.8)	1.15 (0.97–1.38)	
SUCLG2-rs2363712	*IDH3A*-rs11555541				-0.282 (-0.508--0.055)
TC+CC	AA	1,038 (14.8)	305 (13.0)	1.00 (reference)	
TT	AA	780 (11.1)	295 (12.6)	**1.27 (1.06-1.53)**	
TC+CC	AC+CC	2,784 (39.8)	955 (40.7)	1.16 (1.00-1.35)	
TT	AC+CC	2,399 (34.3)	792 (33.7)	1.12 (0.96-1.30)	

AP denotes attributable proportion due to interaction, ORs odd ratios, 95% CIs 95% confidence intervals, SNP single nucleotide polymorphism.

Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 14. Continued.

Gene-SNP	Gene-SNP	Controls, n(%)	Cases, n(%)	ORs (95% CIs)	AP (95% CIs)
SUCLG2-rs2363712	*IDH3A*-rs17674205				-0.496 (-0.866--0.126)
TT	TT	2,651 (38.1)	878 (37.8)	1.00 (reference)	
TC+CC	TT	3,126 (45.0)	1,074 (46.2)	1.04 (0.94-1.15)	
TT	TC+CC	507 (7.3)	196 (8.4)	1.17 (0.98-1.41)	
TC+CC	TC+CC	666 (9.6)	176 (7.6)	**0.81 (0.68-0.97)**	
DLAT-rs10891314	*IDH3A*-rs11555541				-0.288 (-0.530--0.046)
GG	AA	703 (10.1)	223 (9.6)	1.00 (reference)	
GA+AA	AA	1,104 (15.9)	374 (16.0)	1.07 (0.88-1.30)	
GG	AC+CC	2,051 (29.5)	773 (33.1)	**1.19 (1.01-1.42)**	
GA+AA	AC+CC	3,099 (44.5)	965 (41.3)	0.98 (0.83-1.16)	

AP denotes attributable proportion due to interaction, ORs odd ratios, 95% CIs 95% confidence intervals, SNP single nucleotide polymorphism.

Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 15. Odds ratios and 95% confidence intervals for the combined effect of the SNPs within the Citric acid cycle pathway on the risk for the cancer of rectum, showing the 95% CIs of AP not containing zero.

Gene-SNP	Gene-SNP	Controls, n(%)	Cases, n(%)	ORs (95% CIs)	AP (95% CIs)
SDHC-rs17395595	*IDH3A*-rs11555541				-0.341 (-0.672--0.010)
AG+GG	AA	258 (7.2)	68 (5.7)	1.00 (reference)	
AA	AA	644 (18.0)	239 (19.9)	**1.42 (1.04-1.93)**	
AG+GG	AC+CC	707 (19.8)	238 (19.8)	1.29 (0.95-1.75)	
AA	AC+CC	1,966 (55.0)	656 (54.6)	1.28 (0.96-1.69)	
SUCLG2-rs902320	*SDHA*-rs6962				-0.390 (-0.774--0.006)
GA+AA	GG	1,273 (35.5)	395 (32.9)	1.00 (reference)	
GG	GG	1,449 (40.4)	500 (41.6)	1.12 (0.96-1.30)	
GA+AA	GT+TT	392 (10.9)	158 (13.1)	**1.30 (1.04-1.62)**	
GG	GT+TT	472 (13.2)	149 (12.4)	1.02 (0.82-1.26)	
SUCLG2-rs902321	*ACO1*-rs7042042				-0.431 (-0.784--0.078)
TG+GG	GG	957 (26.7)	301 (25.2)	1.00 (reference)	
TT	GG	541 (15.1)	211 (17.6)	**1.25 (1.01-1.53)**	
TG+GG	GA+AA	1,312 (36.7)	456 (38.1)	1.11 (0.94-1.32)	
TT	GA+AA	768 (21.5)	228 (19.1)	0.95 (0.78-1.16)	
SUCLG2-rs2363712	*ACO1*-rs7042042				-0.368 (-0.681--0.054)
TC+CC	GG	836 (23.4)	258 (21.5)	1.00 (reference)	
TT	GG	663 (18.5)	256 (21.3)	**1.25 (1.02-1.53)**	
TC+CC	GA+AA	1,106 (30.9)	385 (32.0)	1.13 (0.94-1.35)	
TT	GA+AA	975 (27.2)	303 (25.2)	1.01 (0.83-1.22)	

AP denotes attributable proportion due to interaction, ORs odd ratios, 95% CIs 95% confidence intervals, SNP single nucleotide polymorphism.

Bold font indicates the statistical significance with a p-value of less than 0.05.

Table 15. Continued.

Gene-SNP	Gene-SNP	Controls, n(%)	Cases, n(%)	ORs (95% CIs)	AP (95% CIs)
SDHA-rs34511054	*ACLY*-rs2304497				-0.704 (-1.362--0.047)
GA+AA	GG	314 (8.8)	93 (7.8)	1.00 (reference)	
GG	GG	2,398 (66.9)	830 (69.2)	1.17 (0.92-1.49)	
GA+AA	GA+AA	93 (2.6)	43 (3.6)	**1.56 (1.02-2.40)**	
GG	GA+AA	777 (21.7)	234 (19.5)	1.01 (0.77-1.33)	

AP denotes attributable proportion due to interaction, ORs odd ratios, 95% CIs 95% confidence intervals, SNP single nucleotide polymorphism.

Bold font indicates the statistical significance with the p-value of less than 0.05.

5. Discussion

In this study, the associations between polymorphisms on the citric acid cycle and colorectal cancer were evaluated in UK populations. The interaction between the citric acid cycle marker and the contributors of energy balance, including obesity, physical activity, and energy intake, on the risk of colorectal cancer, was examined. Furthermore, the SNP-SNP interactions for the risk of colorectal cancer were assessed.

Significant effects for interactions of the citric acid cycle SNPs with obesity and physical activity were observed, although the significant main effect of the citric acid cycle SNPs for colorectal cancer was not found in the present study. Figures Figure 6 and Figure 7 show obesity and physical activity for colon and rectal cancer risk by noncarrier and carrier of the minor allele of SNPs, which had significant interaction with obesity. Figure 8 presents a significant combined effect of pairwise citric acid cycle SNPs for colon cancer.

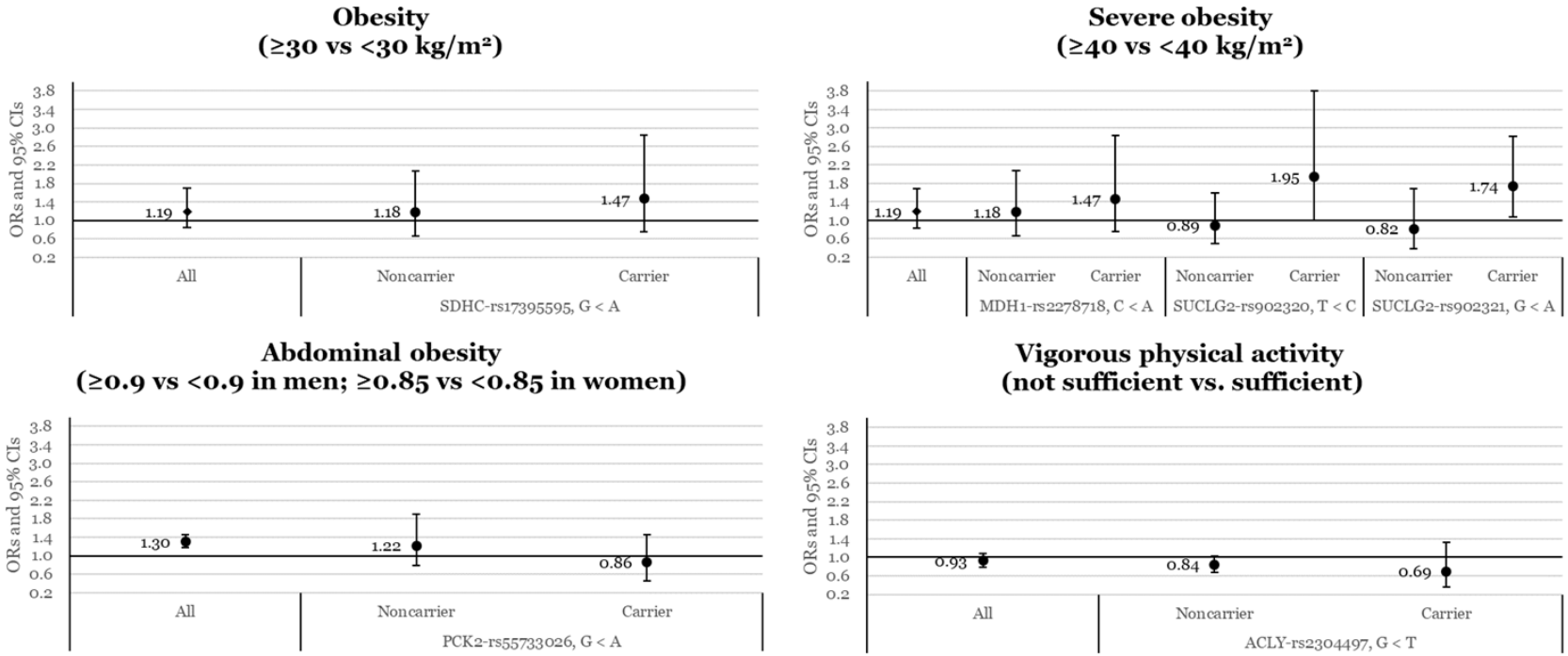


Figure 6. Odds ratios (ORs) and 95% confidence intervals (95% CIs) of obesity and physical activity for colon cancer risk by noncarrier and carrier of minor allele of SNPs, which had significant interaction with environmental factor

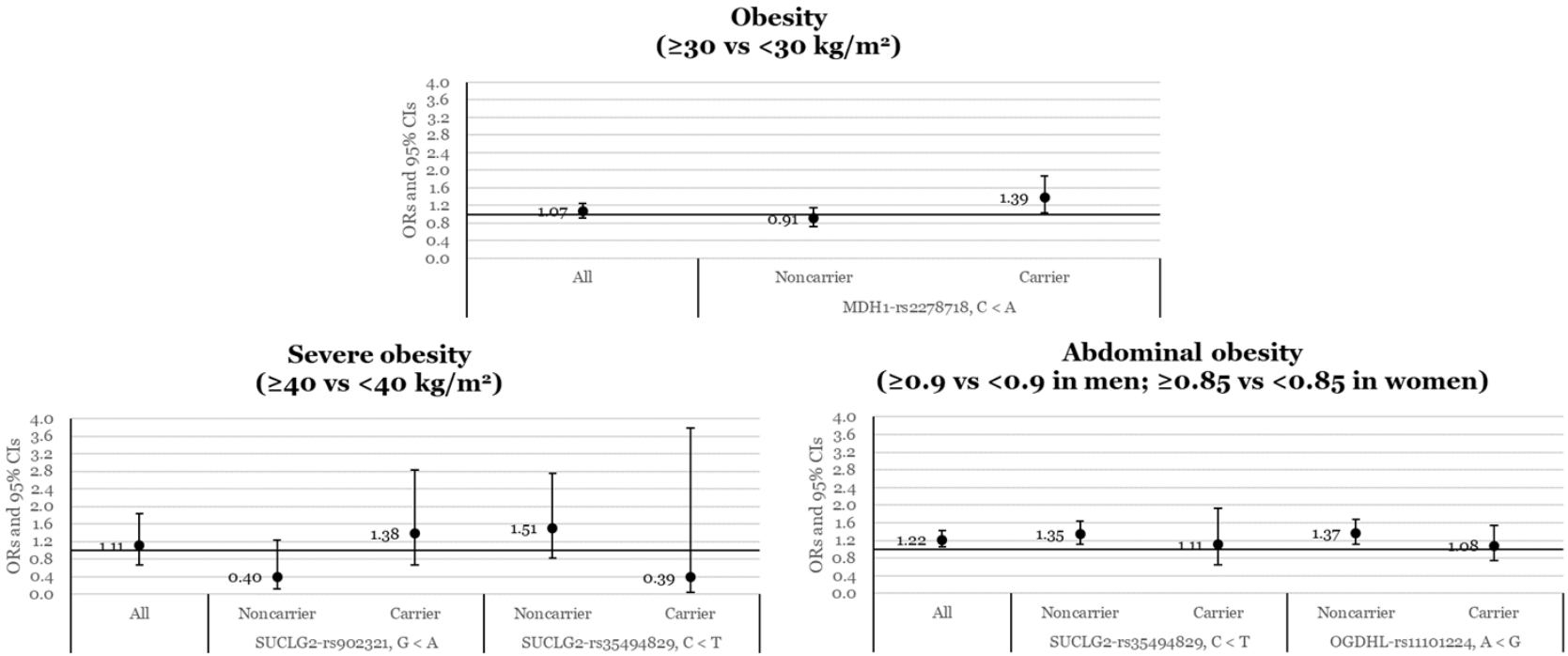


Figure 7. Odds ratios (ORs) and 95% confidence intervals (95% CIs) of obesity for rectal cancer risk by noncarrier and carrier of the minor allele of SNPs, which had significant interaction with an environmental factor

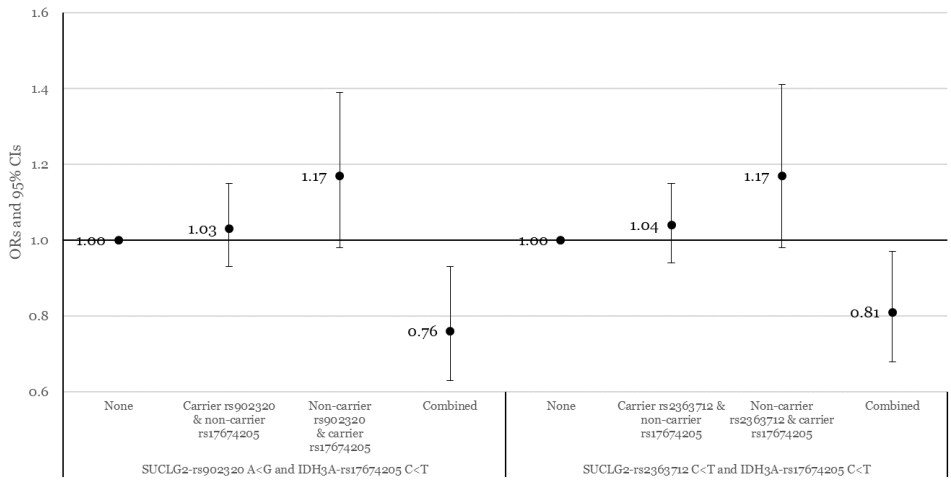


Figure 8. Odds ratios (ORs) and 95% confidence intervals of the significant combined effect of pairwise citric acid cycle SNPs for colon cancer

5.1. Previous studies on polymorphisms of the citric acid cycle

Previous studies on the association between SNPs in the gene encoding the component of the citric acid cycle and any cancer are described below.

Succinate dehydrogenase complex subunit C (SDHC)

The SDHC gene encodes one of four nuclear-encoded subunits comprising succinate dehydrogenase (SDH) enzyme, which links the citric acid cycle to oxidative phosphorylation within the mitochondria. Dysfunction of the electron transport chain due to defects in SDH subunits B, C, or D has been found in patients with gastrointestinal stromal tumors. The results from previous studies also reported that the expression of SDHC was reduced in tumor tissues [64-66]. Alteration on the SDHC gene leads to reduced SDH activity, which increases the levels of mitochondrial succinate and then increases mitochondrial reactive oxygen species [66].

Recent studies have suggested that the consequences of the dysfunctions in

the genes encoding the component of SDH enzyme and fumarate hydratase (FH) to mitochondrial dysfunction and cancers were linked [67] with the dysfunctional cell signaling via oncometabolites including succinate and fumarate [68], and there would be the similarity between the phenotypes of cancer with these mutations [69]. However, contrary to expectations, the interaction between SNPs in the *SDHC* and *FH* gene in colorectal cancer was not found in the present study.

Studies on the association of SNPs in the SDHC gene were conducted for the prognosis of patients with colorectal cancer[70] and hepatocellular carcinoma [71]. The significance associations were shown rs12064957 (1.36 [1.06–1.74]) for overall survival in the additive model, and rs413826 for overall survival and recurrence-free survival (0.61 [0.47–0.79] and 0.73 [0.58–0.91], respectively) in the additive model [70]. Rs3935401 in the 3' untranslated region of *SDHC* exhibited a significant association with OS in hepatocellular carcinoma patients ($p < 0.001$) [71].

Fumarate hydratase (FH)

Rs12071124 in the *FH* gene exhibited borderline significant association with overall survival and significant association with recurrence-free survival among

patients with colorectal cancer [70].

Isocitrate dehydrogenase NADP(+) 1 (IDH1)

Rs12478635 in *IDH1* showed significant associations with death risk in hepatocellular carcinoma patients (HR [95% CIs]; 1.87 [1.27–2.75] in the recessive model) in a cohort study comprised Han Chinese patients [72].

Pyruvate dehydrogenase E1 subunit beta (PDHB)

The PDHB gene encodes the component of the enzyme, which catalyzes the conversion of pyruvate to acetyl coenzyme A and carbon dioxide and provides the primary link between glycolysis and the citric acid cycle. Association between *PDHB* SNPs and colorectal cancer could be supported by a previous study exhibiting the downregulated citric acid cycle and upregulated glucose uptake and lactate production in the colorectal cancer cell with the overexpression of miRNA, which targets the 3′ UTR of *PDHB* mRNA [73].

Aconitase 1 (ACO1)

Rs7874815 in the ACO1 gene have been evaluated for survival among patients with pancreatic cancer in the pooled analysis with Health Professionals

Follow-up Study, Nurses' Health Study (NHS), Physicians' Health Study, and Women's Health Initiative–Observational Study [74]. rs7874815 in the ACO1 gene were associated with survival among patients with pancreatic cancer (hazard ratio [95% CIs] for death per minor allele, 1.37 [1.16–1.61]).

Oxoglutarate dehydrogenase-like (OGDHL)

The OGDHL gene encodes the protein, which is a component of the multi-enzyme oxoglutarate dehydrogenase (OGDH) complex. Previous studies have reported the frameshift mutations of the OGDH gene in colorectal cancer tissue [75], and the OGDHL gene modifying the NF-κB function, which is activated by a variety of proinflammatory cytokines, DNA damage, and free radicals, through increased AKT signaling in the cervical cancer cell [76].

Dihydrolipoamide S-acetyltransferase (DLAT)

The DLAT gene encodes the component E2 of the pyruvate dehydrogenase complex, which resides in the inner membrane of the mitochondria and catalyzes the conversion of pyruvate, which is formed from the breakdown of carbohydrates

to acetyl coenzyme A. Previous studies have reported the association of the DLAT gene with obesity [77] and diabetes mellitus [78].

Succinate dehydrogenase complex subunit D (SDHD)

rs10789859, rs544184, and rs7121782 in the SDHD gene have been evaluated among patients with colorectal cancer[70]. rs544184 and rs7121782 showed significant association with overall survival (HR [95% CIs]; 1.52 [1.05–2.19] and 1.49 [1.04–2.14] in the additive model, respectively). rs10789859, rs544184, and rs7121782 exhibited a significant association with recurrence-free survival (HR [95% CIs]; 1.29 [1.08–1.55], 1.31 [1.08–1.58] and 1.29 [1.07–1.55] in the additive model).

Dihydrolipoamide S-succinyltransferase (DLST)

rs732765 in DLST have been reported to be associated with the prognosis in the advanced non-small cell lung cancer [79]. rs732765 exhibited significance association in the additive model (AA vs AG vs GG, 1.00 vs 1.58 [1.23–2.02] vs 2.19 [1.40–3.43]) and the dominant model (AA vs AG+GG, 1.00 vs 1.66 [1.31–2.10]).

Isocitrate dehydrogenase NADP(+) 2 (IDH2)

It has been reported SNPs in the IDH2 gene on cancer outcome in a cohort [72], case-control [80], and *in silico*[81] studies. rs11632348 in the IDH2 gene exhibited significant associations with death risk in hepatocellular carcinoma patients in the recessive model (HR [95% CIs]; 1.87 [1.27-2.75]) in a cohort study [72]. rs11540478 has been evaluated to be associated with lung cancer risk in a case-control study [80]. Lung cancer patient carriers of rs11540478 TT and CT exhibited higher risk than CC carriers (OR [95% CIs]; 1.44 [1.04–2.00]). NPS in the IDH2 gene has been evaluated using in silico and eQTL analyses in esophageal tissues [81]. rs11630814 as eQTLs and rs4561444 as the functional variants in high linkage disequilibrium were identified.

ATP citric lyase (ACLY)

The ACLY gene has been evaluated in the prognosis and survival of hepatocellular carcinoma[82] and colorectal cancer[83] in the Chinese population. rs2304497 and rs9912300 in *ACLY* showed significant associations with the risks of death (HR [95% CIs]; 0.47 [0.24–0.90] and 0.59 [0.37–0.92], respectively) and

recurrence (0.46 [0.24–0.86] and 0.54 [0.35–0.83], respectively) in patients with stage III + IV of colorectal cancer [83]. rs9912300 in *ACLY* was significantly associated with the overall survival of hepatocellular carcinoma patients only with higher serum alpha-fetoprotein (AFP) level (HR, [95% CIs]; homozygous wild genotype with higher AFP level, 1.46 [1.10–1.95]; variant-containing genotype with higher AFP level, 1.62 [1.17–2.24]; variant-containing genotype with lower AFP level, 1.31 [0.92–1.86] than the homozygous wild genotype with lower AFP level)[82].

5.2. Mechanisms of the citric acid cycle for colorectal cancer

The underlying knowledge of the association between the genes encoding the enzymes of the citric acid cycle and cancer has usually been described as the Warburg effect [84], referring to the phenomenon that occurs in most cancer cells where instead of generating energy with the pyruvate from a high rate of glycolysis undergoes lactic acid fermentation in the cytosol even when oxygen is sufficient [85,86]. In this study, the association between *SUCLG2* rs35494829 and colon cancer (ORs [95% CIs] per increment of the minor allele, 0.82 [0.74–0.92]) was found with statistical significance. These results can be supported by few studies on succinate, which were catalyzed by succinyl-CoA ligase, as an intermediate in cancer metabolism. Results from an in vitro study have suggested that the accumulation of succinate leads to the oncogenic signal via HIF-1α regulatory pathway [87].

Results from the present study shows the significant interactions of *SDHC*, *MDH1*, *SUCLG2, PCK2,* and *ACLY* with obesity, energy intake, and physical

activity for colon cancer, and the interactions of *MDH1*, *SUCLG2,* and *OGDHL* with only obesity on rectal cancer. The interactions with the contributors of energy balance could be explained by mitochondrial dynamics to adapt energy demand and nutrient supply via changes of the mitochondrial morphology [21]. When the energy demand increased, such as physical activity and starvation, mitochondrial elongation, and aerobic respiration, coupled to ATP synthesis, were observed [88,89]. Mitochondrial fragmentation and decreased coupling to ATP synthesis were observed, and the excess energy was consumed in the form of thermogenesis when the energy supply increased, such as high levels of nutrients and obesity [90,91].

The interaction between the citric acid cycle and obesity in colorectal cancer also can be explained by insulin resistance. Insulin resistance is characterized by the inability to effectively manage glucose balance in terms of cellular and metabolism, and mutually influenced by obesity [92]. Furthermore, insulin resistance has been associated with colorectal cancer as well as obesity [93]. A previous review article has suggested that reduced flux of the citric acid cycle can lead to type 2 diabetes mellitus via insulin resistance [94]. It is consistent with the downregulated

citric acid cycle genes among men with obesity compared to men with lean [95].

6. Conclusions

This study found a significant association between *SUCLG2*-rs35494829 and colon cancer. The significant interaction of *SDHC*-rs1735595 with obesity for colon cancer was also shown. To our knowledge, this is the first study to evaluate the citric acid cycle SNPs as colorectal cancer susceptibility loci and their interactions with lifestyle factors for colorectal cancer. Furthermore, this study selected the citric acid cycle SNPs, which were nonsynonymous SNPs or SNPs at a splicing site, as a functional candidate locus of the citric acid cycle for colorectal cancer.

The present study has several limitations. The external validity remained inconclusive since the replication study has not been conducted. The results of this study could not be compared nor supported with those of previous studies. Additionally, statistical evaluation of G×E and G×G interactions may be insufficient to account for complex mechanisms of the citric acid cycle. Although we anticipated providing clues to the etiology in cancer development related to energy metabolism through the results of the present study based on a large

population, the causality remains inconclusive. Thus, further studies are necessary to validate the associations exhibited in this study and to identify precise mechanisms considering the potential confounders. These findings may provide new insights into the genetic susceptibility and molecular mechanisms of obesity and the citric acid cycle in colorectal cancer.

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국문 초록

시트르산 회로 단일 염기 다형성 및 환경요인 간 상호작용과 대장암 발생 위험 탐색

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대장암은 세계적으로 흔한 암종이다. 대장암 발생의 위험요인으로는 비만, 신체활동 감소 등이 있고, 이들은 에너지 균형에 크게 기여하는 요인이기도 하다. 본 연구는 세포 수준에서 에너지 대사에 중심적인 역할을 하는 미토콘드리아의 유전적 다형성으로서 에너지 대사의 개별 차이에 대한 설명을 강화하기 위해 미토콘드리아 시트르산 사이클의 유전적 변이와 대장암 사이의 연관성을 평가하는 것을 목표로 한다. 대장암 발생 위험에 대한 시트르산 사이클의 유전자에 있는 단일 염기 다형성(single nucleotide polymorphism, SNP)와 비만, 신체 활동, 에너지 섭취 간 상호작용도 평가하였다. 또한, 시트르산 사이클의

SNP-SNP 간 상호작용도 평가하였다.

본 연구는 UK Biobank 연구의 데이터를 사용하였다. 연구 참여자들은 3,523명의 대장암 환자와, 환자군에 대해 매칭한 10,522명의 대조군을 포함한다. 비만은 체질량지수 (body mass index, BMI)와 허리 대 엉덩이 둘레 비 (waist to hip ratio, WHR)를 사용하여 정의되었다. 참가자들의 BMI가 30보다 크거나 같으면 비만으로, BMI가 40보다 크면 중증 비만으로 분류됐다. 복부비만은 WHR이 0.9 이상인 남성, 0.85 이상인 여성으로 정의하였다. 에너지 섭취량이 권고된 양보다 초과된 참가자는 여성의 경우 하루 에너지 소비량이 2,000 kcal 이상, 남성은 2,500 kcal 이상인 것으로 정의하였다. 일주일에 150분 이상의 중강도 신체활동 또는 75분 이상의 고강도 신체활동을 수행한 연구대상자는 건강이 증진될 수준의 신체활동을 한 것으로 분류되었다. 대장암에 대한 시트르산 사이클 SNP의 effect size는 codominant, dominant 및 additive model을 가정하여 평가하였다. 대장암과 직장암에 대한 오즈비(odds ratio, OR)와 95% 신뢰 구간(95% confidence intervals, 95% CIs)은 조건부 로지스틱 회귀 모형을 사용하여 추정하였다. 다중 비교를 보정하기 위해 false discovery rate를 사용하다.

SUCLG2-rs35494829는 dominant model (OR [95% CI]; 0.82

[0.74–0.92]) 및 additive model (0.82 [0.74–0.92])에서 대장암의 위험 감소와 연관성이 있었다. 다중 비교에 대한 보정을 한 후에도 *SUCLG2*–rs35494829와 대장암 사이의 연관성은 통계적으로 유의했다 (p=0.0206). 대장암에 대한 *SDHC*–rs17395595와 비만 사이의 교호작용이 발견되었으며($p_{interaction}$=0.0023), 다중 비교를 보정한 후에도 이 교호작용은 통계적으로 유의했다 ($p_{interaction}$=0.047). 시트르산 회로의 SNP 간 교호작용은 교호작용으로 인한 기여 분율 (attributable proportion of disease due to interaction with both exposures, AP)을 계산 평가했다. 대장암과 직장암에 대한 시트르산 사이클 SNP 간 교호작용으로 인한 기여분율이 음의 값인 것을 관찰할 수 있었지만, 통계적으로 유의하지 않았다.

본 연구에서 *SUCLG2*–rs35494829와 대장암 사이의 유의미한 연관성을 발견할 수 있었다. 또한, 대장암에 대한 *SDHC*–rs17395595와 비만 사이의 유의한 상호작용도 관찰할 수 있었다. 이 연구의 결과를 통해, 대장암 발생에 대한 비만과 시트르산 회로의 분자 메커니즘에 대한 새로운 근거를 제시하고자 한다.