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Review Article

Lack of association between LCT-13910 C/T (rs4988235) polymorphism and risk of colorectal cancer: evidence from case-control studies

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Abstract: Objective: Several published studies have investigated the association between 13910 C > T (rs4988235) polymorphism in the lactase (LCT) gene and colorectal cancer risk. However, results remain controversial. This meta-analysis was performed, aiming to systematically unravel this inconsistency. Methods: Eligible studies reporting the association between LCT rs4988235 polymorphism and colorectal cancer susceptibility were included from PubMed, Embase, Web of Science, and Cochrane Library. Odds ratios (OR) with 95% confidence intervals (CIs) were used to calculate the strength of association. Publication bias detection was conducted using Begg's test. Results: A total of five case-control papers, involving seven studies, were included and reported a total of 1,972 cases and 2,230 controls. However, no significant association was uncovered between LCT rs4988235 polymorphism and colorectal cancer susceptibility in the overall combined population under the five genetic models (Allelic model: C vs. T, p = 0.939; homozygous model: CC vs. TT, p = 0.767; heterozygous model: CC vs. CT, p = 0.214; dominant model: CT + TT vs. CC, p = 0.164; recessive model: TT vs. CC + CT, p = 0.542). Conclusions: The present meta-analysis suggests no association between LCT rs4988235 polymorphism and colorectal cancer susceptibility. Results indicate that LCT rs4988235 polymorphism is not a risk factor for colorectal cancer. Given the limited ethnic groups and small sample sizes, future studies are required to further validate the association.

Keywords: Lactase, rs4988235, colorectal cancer, polymorphism, meta-analysis

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related deaths in Western countries [1-4]. CRC is a complex disease process involving dietary, genetic, and environmental factors. Epidemiologic studies have indicated that milk, mainly calcium, plays a preventive role in the progress of CRC [5, 6]. It has been reported that calcium intake and absorption are obviously decreased in patients with lactose intolerance [7, 8]. Lactose is the main sugar of milk. It is hydrolyzed to galactose and glucose with the help of the lactase enzyme in the intestinal wall. Downregulation of lactase activity results in failure to absorb and digest lactose, causing symptoms of lactose intolerance, such as bloating, flatulence, abdominal pain, and diarrhea [9].

The lactase gene (LCT) codes an important enzyme, lactase-phlorizin hydrolase (LPH), that contributes to the digestion of milk [10]. Recently, the single nucleotide polymorphism rs4988235, residing in the minichromosome maintenance-6 gene upstream, the LCT-encoding gene carrying a change C > T, was found to affect regulation of LCT gene expression and adult-type hypolactasia [11-13]. Recent studies have presented a positive association between the CC genotype and an increased CRC risk among Finnish populations [14]. This association, however, was not con-

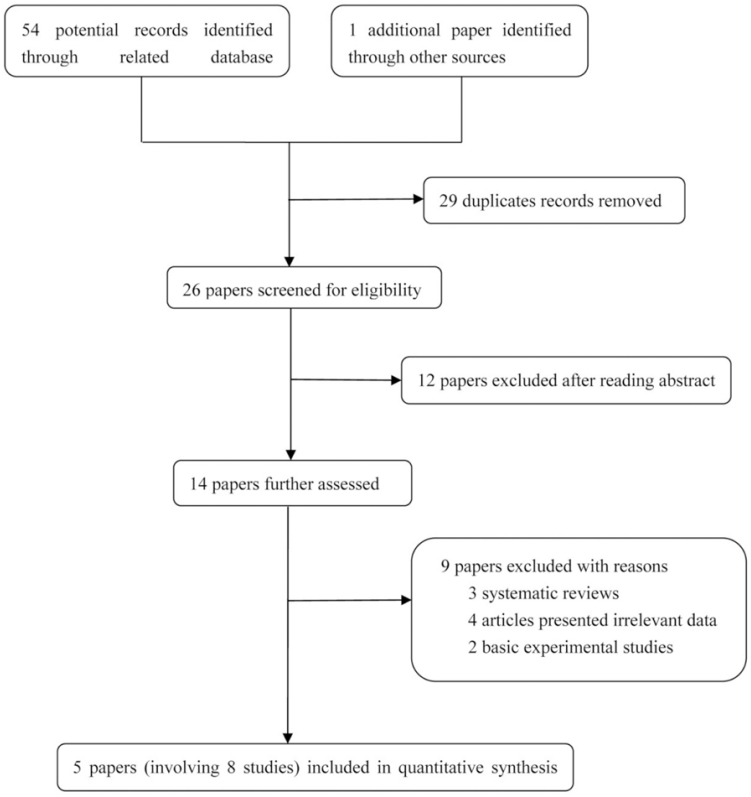

Figure 1. Detailed procedures of the literature search.

firmed in British, Hungarian, Spanish, Turkish, or Italian patients [10, 14-17]. Thus, the roles of 13910 C > T polymorphism in CRC remain unclear due to limited sample sizes and varied genetic backgrounds. Therefore, the present meta-analysis was conducted to investigate the relationship between rs4988235 polymorphism and colorectal cancer risk.

Materials and methods

Search strategy

A comprehensive search was performed to identify relevant publications concerning the association between colorectal cancer risk and rs4988235 polymorphism. Four databases were searched (PubMed, Embase, Web of Science, and Cochrane Library) from inception to July 15, 2018. Search terms included "colorectal cancer", OR "colorectal neoplasms", OR "colorectal tumor", OR "colorectal carcinoma", AND "polymorphism", OR "variant", OR "single nucleotide polymorphism", OR "mutations", OR "genotype", OR "SNP", OR "Alleles", AND "13910 C > T", OR "13910 C/T", OR "Lactase", OR "LCT", OR "rs4988235", without any limitations imposed. Moreover, publications listed in references were screened carefully to identify potential articles discussing this topic.

Inclusion and exclusion criteria

Studies were included if they met the following criteria: (1) Original papers designed in a case-control format; (2) Studies assessing the relationship between LCT-13910 C/T (rs4988235) polymorphism and colorectal cancer susceptibility; (3) Availability of data regarding total number of cases and controls, as well as number of cases and controls with C/C, C/T, and T/T genotypes; (4) At least two comparison groups (cancer group and control group); and (5) Studies focusing on humans. Studies were excluded if they were: (1) Studies without a control group, review articles, case reports, and comments; (2) Studies without raw data regarding the LCT-13910 C/T (rs4988235) polymorphism; and (3) Animal studies. If there were duplicate reports, the latest studies were retained.

Data extraction

Two experienced investigators, independently, performed data extraction according to a predefined data extraction form. Any disagreements were resolved by discussion including all authors. Recorded information included first author's surname, country in which the study was performed, year of publication, ethnicity of subjects, total number in case and control groups, as well as number of cases and controls with C/C, C/T, and T/T genotypes, genotyping method, and *P* values for Hardy-Weinberg equilibrium (HWE).

Quality assessment

Two investigators, independently, performed quality assessment using the 9-star Newcastle-Ottawa Scale (NOS) [18], one of the widely used rating systems for assessing quality of observa-

Table 1. Characteristics of studies included in the meta-analysis

First author	Year	Country	Ethnicity	Source of controls	Genotyping method	Number (case/control)	HWE	NOS	OR	Male/Female
Gençdal	2017	Turkey	Turkish	HB	PCR and minisequencing	44/48	0.326	8	NP	43/49
Bácsi	2008	Hungary	Hungarian	HB	TaqMan real time-PCR	278/260	0.2618	7	1.2	120/95
Piepoli	2007	Italy	Italian	HB	PCR-RFLP	124/254	0.3392	8	NP	263/115
Rasinperä (I)	2005	Finland	Finnish	HB	PCR and minisequencing	773/773	0.3653	7	1.4	455/425
Rasinperä (II)	2005	UK	British	HB	PCR and minisequencing	283/363	0.3457	7	1.05	NP
Rasinperä (III)	2005	Spain	Spanish	HB	PCR and minisequencing	163/221	0.064	7	0.81	NP
Tarabra	2010	Italy	Italian	HB	TaqMan real time-PCR	306/311	0.3099	8	1.04	330/287

HWE: Hardy-Weinberg equilibrium; HB: hospital based; NOS = Newcastle-Ottawa Scale; NP: Not Provide; OR: Odds Risk.

Table 2. Polymorphism genotype distribution and allele frequency in cases and controls

First author	Genotype (N)								Allele frequency (N)			
	Case (N/%)				Control (%)				Case		Control	
	Total	CC	CT	TT	Total	CC	CT	TT	C	T	C	T
Gençdal	44	38 (86.36)	5 (11.36)	1 (2.28)	48	40 (83.33)	7 (17.5)	1 (2.5)	81	7	87	9
Bácsi	278	113 (40.65)	119 (42.81)	46 (16.54)	260	95 (36.53)	117 (45)	48 (18.46)	345	211	307	213
Piepoli	124	99 (79.84)	25 (20.16)	0 (0)	254	214 (84.25)	37 (14.57)	3 (1.18)	223	25	465	43
Rasinperä (I)	773	182 (23.54)	383 (49.55)	208 (26.91)	773	139 (17.98)	392 (50.71)	242 (31.31)	747	799	670	876
Rasinperä (II)	283	26 (9.19)	100 (35.33)	157 (55.48)	363	32 (8.81)	139 (38.29)	192 (52.89)	152	414	203	523
Rasinperä (III)	163	52 (31.9)	77 (47.24)	34 (20.86)	221	81 (36.65)	116 (52.49)	24 (10.86)	181	145	278	164
Tarabra	306	190 (62.09)	101 (33.01)	15 (4.9)	311	196 (63.02)	98 (50)	17 (5.47)	481	131	490	132

tional studies in a meta-analysis. The NOS involves three aspects, case and control selection, comparability, and exposure. Each aspect consists of four, two, and three items, respectively. Each item values one score, with nine scores in total. NOS scores of each study equal with greater than 6 stars was regarded as high quality.

Statistical analysis

Statistical analyses were conducted with STATA 12.0 software (StataCorp, College Station, TX, USA). Association strength between LCT-13910 C/T (rs4988235) polymorphism and colorectal cancer susceptibility was assessed by calculating ORs along with 95% CI. Pooled ORs were measured using the Z-test. Hardy-Weinberg equilibrium (HWE) for each single nucleotide polymorphism was tested by the χ^2 test. ORs were combined using either a random-effects model or a fixed effects model, as previously described [19]. *P*-values less than 0.05 indicate statistically significant differences. Heterogeneity among studies was determined using the Q-test and I-square statistic tests, with $I^2 < 50\%$ indicating insignificant differences. Sensitivity analysis was also carried out to test the stability of results. Begg's funnel plot test was used to assess potential publication bias, with $P < 0.05$ indicating statistical significance.

Results

Characteristics of included studies

The initial search yielded a combined 55 references through related electronic databases. A flow chart of the study selection process is shown in **Figure 1**. A total of 5 original articles, involving seven studies, met the inclusion criteria for this meta-analysis. Detailed characteristics of the seven included studies are shown in **Table 1**. All study subjects were in Western countries. Four studies were performed using polymerase chain reaction-restriction (PCR) and mini-sequencing, two used PCR TaqMan, and one used PCR fragment length polymorphism (PCR-RFLP). Frequencies of each genotype and allele, with their HWE values, are listed in **Table 2**. The analyzed single nucleotide polymorphism was within HWE across all included studies. NOS score results ranged from 7 to 8, with an average of 7.42, indicating that the methodological quality of the seven selected studies was generally reliable (**Table 1**).

Table 3. Meta-analysis of the association between miR-146a rs2910164 polymorphism and cancer susceptibility

SNP	Association results		Heterogeneity		
	OR (95% CI)	$P_{(Z-t)}$	$P_{(Q-t)}$	I^2 (%)	Model
C vs. T	0.99 (0.85-1.16)	0.939	0.058	50.8	Random effects
CC vs. TT	0.95 (0.66-1.36)	0.767	0.164	53.3	Random effects
CC vs. TC	0.91 (0.78-1.06)	0.214	0.396	4.0	Fixed effects
TT vs. CC + TC	0.95 (0.82-1.11)	0.542	0.064	49.6	Fixed effects
CT + TT vs. CC	0.90 (0.78-1.04)	0.482	0.197	30.3	Fixed effects

OR = odds ratios, SNP = single nucleotide polymorphism, $P_{(Z-t)}$ value for association test, $P_{(Q-t)}$ value for heterogeneity test.

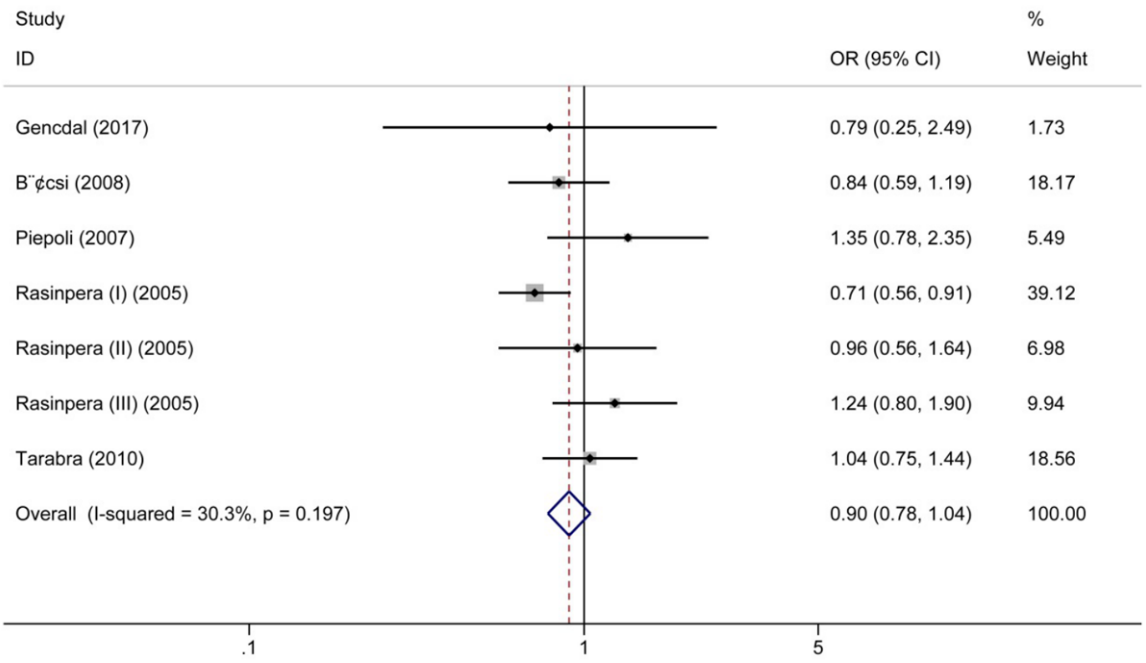

Figure 2. Forest plot of pooled odds ratios of the association of LCT-13910 C/T (rs4988235) polymorphism with colorectal cancer susceptibility in the dominant model.

Meta-analysis results

A total of seven studies, involving 1,972 cases and 2,230 controls, were included. All studies were conducted among European populations. Overall, there were no significant statistical differences between the polymorphism and colorectal cancer in the five models (allele model: C vs. T, OR = 0.99, 95% CI: 0.85-1.16; homozygous model: CC vs. TT, OR = 0.95, 95% CI: 0.66-1.36; heterozygous model: CC vs. TC, OR = 0.91, 95% CI: 0.78-1.06; recessive model: TT vs. CC + TC, OR = 0.95, 95% CI: 0.81-1.11; dominant model: CT + TT vs. CC, OR = 0.90, 95% CI: 0.78-1.04). The main results of this meta-analysis are listed in **Table 3**.

Figure 2 shows the forest plot of pooled odds ratios of the association of LCT-13910 C/T (rs4988235) polymorphism with colorectal cancer susceptibility under the dominant model.

Subgroup analysis

Subgroup analysis was only available for genotyping methods and NOS scores. Stratified subgroup analysis revealed no obvious association between this polymorphism and colorectal cancer among European populations in the five models (all $p > 0.05$). Subgroup analysis of the association of rs4988235 polymorphism with colorectal cancer under the dominant model is shown in **Table 4**.

Table 4. Subgroup analysis of the association of rs4988235 polymorphism with colorectal cancer under dominant model

Subgroup analysis	N	OR	95% CI	$P_{(Z-t)}$	I^2 (%)	$P_{(Q-t)}$
Overall	7	0.90	0.78-1.04	0.482	30.3	0.197
Genotyping method						
PCR and minisequencing	4	0.89	0.66-1.21	0.452	40.6	0.168
TaqMan and real time-PCR	2	0.94	0.74-1.19	0.621	0	0.381
PCR-RFLP	1	1.35	0.78-2.35	0.287	-	-
NOS score						
Score = 7	4	0.87	0.69-1.11	0.264	40.5	0.169
Score = 8	3	1.09	0.83-1.43	0.529	0	0.619

NOS = Newcastle-Ottawa Scale, OR = odds ratios, CI = confidence intervals, PCR = polymerase chain reaction, RFLP = restriction fragment length polymorphism, N = the number of studies, $P_{(Z-t)}$ value for association test, $P_{(Q-t)}$ value for heterogeneity test.

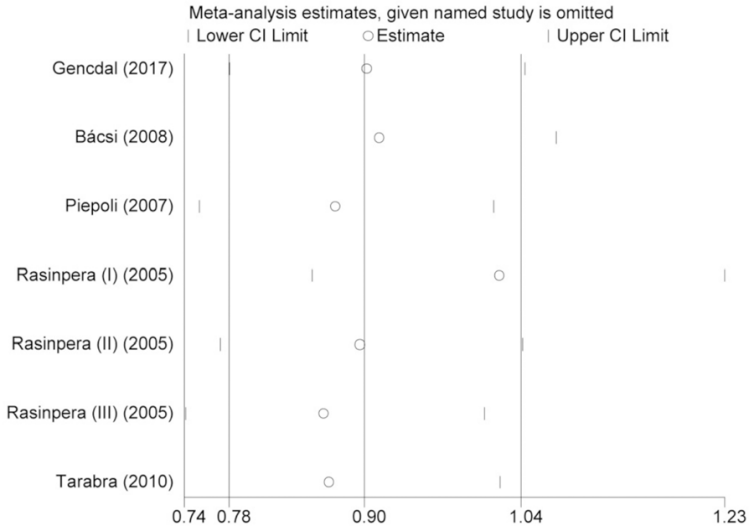

Figure 3. Sensitivity analysis of the association between rs4988235 polymorphism and the risk of colorectal cancer in the dominant model.

Sensitivity analysis

Sequential omission of a single-study was utilized to examine sensitivity in the five models. Pooled OR and 95% CI showed no significant quantitative changes, indicating that pooled results were robust and reliable. Sensitivity analysis concerning the association between rs4988235 polymorphism and risk of colorectal cancer in the dominant model is shown **Figure 3**.

Publication bias

Possible publication bias was estimated using Begg's funnel plot test. No obvious publication bias was found for the association between rs4988235 polymorphism and colorectal cancer susceptibility (dominant model: P = 0.258, **Figure 4**), suggesting that results were statistically robust.

Discussion

Colorectal cancer is a multigenic and multifactorial disease. It is often influenced by *Escherichia coli* infections, environmental factors, and genetic mutations [20, 21]. Many publications have proven that gene polymorphisms contribute to colorectal cancer [22]. Recently, a single nucleotide polymorphism, LCT-13910 C/T (rs4988235), was shown to be associates with lactase persistence [12]. Although the association between rs4988235 polymorphism and colorectal cancer susceptibility has been investigated by several authors, results have been inconclusive. Rasinpera [14] et al. found a positive association between rs4988235 and colorectal cancer risk, with an OR of 1.40 (95% CI: 1.07-1.85). This association was not confirmed in British and Spanish populations, however, with an OR of 1.05 (95% CI: 0.59-1.86) and 0.81 (95% CI: 0.59-1.10), respectively. Another study performed in Italy failed to show a positive link between rs4988235 and colorectal cancer risk, with an OR of 1.041 (95% CI: 0.751-1.442) [10]. A recent study performed among Turkish populations found no association between rs4988235 polymorphism and colorectal cancer risk [17].

Apparently, the variation locates on mini-chromosome maintenance-6 gene, which upstreams the LCT-encoding gene, but not on the LCT-encoding gene. Thus, even if there is an association between rs4988235 and CRC, the risk may result from the malfunction of mini-chromosome maintenance-6 gene. Whether such mutations have loss-of-function effects

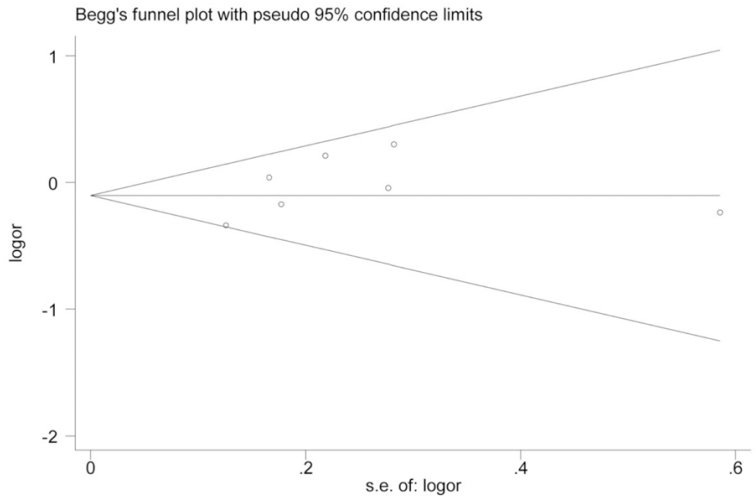

Figure 4. Publication bias detection between rs4988235 polymorphism and colorectal cancer susceptibility in the dominant model.

on the malfunction of minichromosome maintenance-6 gene, which may regulate the LCT gene, requires further investigation. Another possibility is that rs4988235 locates on the regulatory region of LCT gene.

A single study lacks enough statistical power to confirm the relationship between rs4988235 polymorphism and colorectal cancer risks, due to the small sample size. To explore the uncertain association between rs4988235 and risk of colorectal cancer, the present meta-analysis was conducted. This meta-analysis included 1,972 colorectal cancer patients and 2,230 controls, aiming to verify the association between colorectal cancer susceptibility and LCT-13910 C/T (rs4988235) functional polymorphism. Results demonstrated no association between LCT rs4988235 polymorphism and colorectal cancer susceptibility. Results further suggest that LCT rs4988235 polymorphism is not a risk factor for colorectal cancer. Subgroup analysis, with respect to genotyping methods and NOS scores, revealed no obvious association between this polymorphism and colorectal cancer among European populations.

To the best of our knowledge, this is the first meta-analysis evaluating the association between rs4988235 polymorphism and colorectal cancer susceptibility. However, some limitations should be considered when interpreting present results. First, only 7 studies were included in this meta-analysis, which limited further analyses because the original data was unavailable. Further subgroup analyses could not be conducted since the ethnicity was limited to Caucasians. Moreover, this study was unable to adjust for potential confounding effects, such as gender, environmental factors, medication consumption, lifestyle, and other exposure factors, due to limited data. This study failed to verify the results from the level of molecular mechanisms. Data from large multicenter epidemiological studies are necessary to clarify the relationships.

In conclusion, the present meta-analysis, based on seven case-control studies, demonstrated no association between LCT rs4988235 polymorphism and colorectal cancer susceptibility. The rs4988235 polymorphism is not a risk factor for colorectal cancer. However, due to the limited publications included, present results require verification from future studies. More evidence from epidemiologic studies is necessary to validate present results regarding the roles of rs4988235 polymorphism in genetic susceptibility to colorectal cancer.

Disclosure of conflict of interest

None.

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