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Genetic polymorphisms in fatty acid metabolism genes and colorectal cancer

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Colorectal cancer (CRC) is a leading cause of cancer death worldwide. Epidemiological risk factors for CRC included dietary fat intake; consequently, the role of genes in the fatty acid biosynthesis and metabolism pathways is of particular interest. Moreover, hyperlipidaemia has been associated with different type of cancer and serum lipid levels could be affected by genetic factors, including polymorphisms in the lipid metabolism pathway. The aim of this study is to assess the association between single-nucleotide polymorphisms (SNPs) in fatty acid metabolism genes, serum lipid levels, body mass index (BMI) and dietary fat intake and CRC risk; 30 SNPs from 8 candidate genes included in fatty acid biosynthesis and metabolism pathways were genotyped in 1780 CRC cases and 1864 matched controls from the Molecular Epidemiology of Colorectal Cancer study. Information on clinicopathological characteristics, lifestyle and dietary habits were also obtained. Logistic regression and association analysis were conducted. Several *LIPC* (lipase, hepatic) polymorphisms were found to be associated with CRC risk, although no particular haplotype was related to CRC. The SNP rs12299484 showed an association with CRC risk after Bonferroni correction. We replicate the association between the T allele of the *LIPC* SNP rs1800588 and higher serum high-density lipoprotein levels. Weak associations between selected polymorphism in the *LIPC* and *PPARG* genes and BMI were observed. A path analysis based on structural equation modelling showed a direct effect of *LIPC* gene polymorphisms on colorectal carcinogenesis as well as an indirect effect mediated through serum lipid levels. Genetic polymorphisms in the hepatic lipase gene have a potential role in colorectal carcinogenesis, perhaps though the regulation of serum lipid levels.

Introduction

Colorectal cancer (CRC) is a leading cause of death worldwide, with over 1 million of new cases and a half a million of deaths around the world every year (1,2). Risk factors for CRC include advanced age, medical history of benign adenomatous polyps and inflammatory bowel diseases, family history of CRC, low intake of vegetables and fruits and high intake of dietary fat (particularly animal fat) and processed meat (3–6). Chronic consumption of non-steroidal anti-inflammatory drugs, hormone replacement therapy and statins are protective (7,8). The role of other lifestyle factors such as tobacco smoking or alcohol consumption remains inconclusive (9–11).

Dietary fat is a recognized risk factor for CRC. Polymorphisms in genes involved in the regulation of lipid biosynthesis, transport and metabolism are potential susceptibility factors but the effects of these genes in CRC risk may be mediated and modified through their implication in energy balance, obesity or physical activity (12–17).

The aim of the present study is to assess the association between single-nucleotide polymorphisms (SNPs) in lipid metabolism genes, in particular in genes related to fatty acid biosynthesis and metabolism and CRC risk. The potential mediation of serum lipid levels, body mass index (BMI) and dietary fat intake using a comprehensive approach based on structural equation modelling in relation to CRC has been assessed as well. The analyses have been performed within a large population-based case control study conducted in Israel, the Molecular Epidemiology of Colorectal Cancer (MECC) study.

Methods

Patients

The MECC study has already been described in detail (8,18). In brief, it is a population-based case-control study of incident, pathologically confirmed, invasive CRC diagnosed between May 31, 1998, and March 31, 2004, and who lived in a geographically defined area of Northern Israel.

Controls with no prior history of CRC were identified from the same source population using the Clalit Health Services (CHS) database, covering ~70% of the older population. Controls were matched to cases by year of birth, gender, primary clinic location and Jewish or Arab ethnicity. Participants provided written informed consent at the time of enrolment. The Institutional Review Boards at the Carmel Medical Center and the University of Michigan approved all procedures. The study population included 2100 matched pairs. For this analysis, only 1780 cases and 1864 controls with available DNA for genetic analysis will be used. The demographic characteristics of the subjects not included do not differ from those analysed (data not shown).

Life-style information

Participants were interviewed face-to-face by trained monitors with a comprehensive epidemiological questionnaire that assessed demographic information, personal and family history of cancer, reproductive history and medical history, medication use, health habits and a food frequency questionnaire. Patient's weight was recorded by self-report, as estimated 1 year before diagnosis for cases and at the time of interview for controls. BMI was estimated 1 year before the diagnosis from self-reported weight and height.

Comprehensive dietary habits were obtained with the use of a validated food-frequency questionnaire modified for the Israeli diet. Total fat, saturated fat, monounsaturated fat and polyunsaturated fat intakes were estimated from the

questionnaire using local food composition tables. Similar estimates provided total energy consumption. Serum lipid levels at the time to CRC diagnosis were available from electronic medical records of CHS for a subset of the study population. Physical activity was recorded for the longest occupation and also recreational physical exercise. These were combined into an ordinal index ranging from sedentary to intense physical activity.

SNP selection and genotyping

Eight genes (*APOE*, *LIPC*, *LPL*, *CYP1A2*, *ACSL5*, *PPARG*, *PTGS2*) related to lipid metabolism pathways, mainly fatty acid metabolism, were selected for analysis within a larger exploratory project including other hypothesis. Basically, candidate genes were selected from fatty acid biosynthesis and metabolism pathways of the KEGG pathway database (19). Moreover, a literature review was conducted to identify molecular epidemiological studies that analysed the relationship between SNPs in fatty acid metabolism and CRC risk. We searched in Pubmed citation database combinations of the following terms: 'polymorphism or SNP' and 'lipid metabolism or fatty acid metabolism' and 'colon or colorectal cancer'. A total of 30 SNPs in these 8 candidate genes (Table II) were selected as maximally informative htSNPs when the regions were analysed with HaploTagger. SNPs were genotyped using Illumina Beadstation and BeadExpress platforms. Data were analysed using unsupervised Illumina BeadStation SNP calling automated routines, and the distribution of genotypes was compared to those expected from Hardy–Weinberg equilibrium. Genotypes were also confirmed with a second platform in at least 1% of samples.

Statistical methods

Unconditional logistic regression was used to assess the association between genotypes and CRC risk. All models included age and gender to account for the matching design and avoid excluding incomplete pairs due to DNA unavailability or missing values in other variables. Multivariate-adjusted odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated for each genotype compared to those homozygous for the common allele. Also a log-additive model was fitted (trend test). Linear regression was used to assess the associations with the quantitative variables serum lipid levels, BMI and dietary fat intake. In these models, mean differences and 95% CI were calculated. Analysis was performed with the SNPstats (20) web software, including the estimation of haplotypes for multiple SNPs of a gene and SNPassoc library for R package (21). A panel of 300 anonymous SNPs useful for population stratification analysis was analysed in these subjects in relation to other larger genotyping project. No relevant population structure was identified that could not be explained by reported ethnicity. The potential confounding of this variable was rejected after a sensitivity analysis and was not used in the models to maximize efficiency.

A global 5% significance level was desired for the analyses of SNPs. Thus, a P-value < 0.0017 was considered statistically significant following the Bonferroni method to account for the number of tested SNPs as independent hypothesis. All reported P-values are two tailed, adjusted for age, sex and ethnicity but uncorrected for multiple comparisons.

To simultaneously study the associations between SNPs, mediator variables and CRC, we also fitted structural equation models (SEMs) to prespecified path hypothesis. SEM was used to assess the direct effect of genetic polymorphisms on CRC (unexplained by candidate variables), and also their indirect effect, mediated through lifestyle-related factors by including all variables in the same model to assess overall associations. These analyses were performed using structural equation modelling library from R package (21) and MPLUS v. 5.21 (22). SEM was used to test the conceptual model since, in contrast to traditional analytical procedures as linear regression analysis, SEM allows distinguishing between direct and indirect effects and provides information on the degree of fit for the entire model. In SEM, the covariance structure that follows from the proposed model is fitted to the observed covariance. The maximum likelihood estimate method yields estimates of the regression coefficients in the model, standard errors and a overall goodness-of-fit test (23).

Results

The study population included 3644 participants, 1780 cases and 1864 controls, with available data regarding

Table I. Baseline characteristics of cases and controls from the study population

Characteristic	Cases	Controls	P-value
N	1780	1864	
Clinical/baseline characteristics			
Age, mean (SD)	70.21 (11.67)	70.58 (11.67)	0.74
Sex			0.65
Male, *N* (%)	894 (50.22)	931 (49.95)	
Female, *N* (%)	886 (49.78)	933 (50.05)	
Height, mean (SD)	165.78 (8.68)	165.42 (8.90)	0.50
Weight, year before diagnosis, mean (SD)	75.13 (14.45)	73.71 (13.99)	0.0093
BMI, mean (SD)	27.26 (4.63)	26.89 (4.63)	0.037
History of CRC in first-degree relative			0.0019
No, *N* (%)	1571 (89.98)	1722 (92.88)	
Yes, *N* (%)	175 (10.02)	132 (7.12)	
Physical activity index, *N* (%)			0.0000
Sedentary	1231 (69.16)	1105 (59.28)	
Intermediate	311 (17.47)	426 (22.85)	
Active	238 (13.37)	333 (17.86)	
Vegetable intake, mean (SD)	7.47 (4.48)	7.85 (3.69)	0.0022
Regular aspirin intake			
No, *N* (%)	1192 (69.18)	1170 (62.84)	0.0001
Yes, *N* (%)	531 (30.82)	692 (37.16)	
Regular statin intake			
No, *N* (%)	1630 (94.17)	1623 (87.68)	0.0000
Yes, *N* (%)	101 (5.83)	228 (12.32)	
Dietary fat intake[a]	692	1854	
Energy, mean (SD)	1768.15 (1006.97)	1814.12 (898.08)	0.13
	1710	1862	
Total fat, mean (SD)	69.35 (67.48)	66.14 (44.91)	0.0044
Fat density[a]	1687	1854	
Total fat, mean (SD)	0.33 (0.07)	0.32 (0.06)	0.00053
Serum lipids (1 year prior to diagnosis)	272	625	
HDL, mean (SD)	50.03 (12.80)	50.66 (12.11)	0.32
LDL, mean (SD)	122.06 (31.37)	120.78 (29.40)	0.60

[a]Cases with energy >10000 Kcal have been excluded.

Table II. Association (*P*-values[a]) between polymorphisms in fatty acid metabolism genes and CRC risk, serum lipid levels, BMI and fat intake

SNP	CRC	HDL 1 year before	LDL 1 year before	BMI	Fat density[f]	Energy (Kcal)
APOE_rs405509	0.44	0.16	0.62	0.67	0.50	0.0024
APOE_rs439401	0.24	0.49	0.63	0.71	0.034	0.048
APOE_rs7259620	0.66	0.15	0.30	0.30	0.52	0.067
LIPC_rs12913969	0.44	0.29	0.78	0.98	0.48	0.26
LIPC_rs16940302	0.66	0.87	0.69	0.060	0.86	0.31
LIPC_rs16940372	**0.0086**	0.14	0.76	0.63	0.56	0.86
LIPC_rs17190510	0.70	0.29	0.45	0.42	0.36	0.24
LIPC_rs1800588	0.51	**0.025**	0.18	0.92	0.99	0.71
LIPC_rs2099190	0.17	0.36	0.19	0.81	0.57	0.84
LIPC_rs3751542	0.85	0.58	0.82	0.73	0.20	0.68
LIPC_rs4774302	0.14	0.40	0.92	0.18	0.20	0.30
LIPC_rs4775053	**0.0099**	0.92	0.88	0.05	0.84	0.77
LIPC_rs4775072	**0.0028**	0.49	0.11	**0.023**	0.19	0.64
LIPC_rs6083	0.65	0.87	0.92	0.10	0.48	0.22
LIPC_rs634746	0.54	0.75	0.18	0.10	0.90	0.36
LIPC_rs7166788	0.65	0.87	0.80	0.35	0.30	0.63
LIPC_rs7174210	0.17	0.14	0.99	**0.017**[b]	0.50	0.97
LIPC_rs8035006	0.20	0.87	0.33	0.68	0.31	0.35
LIPC_rs9652472	**0.0005**	0.86	0.087	**0.070**	0.55	0.52
LPL_rs268	0.034[c]	—	—	0.72	0.090[c]	—
LPL_rs328	0.97	0.15	0.61	0.55	0.49	0.99
CYP1A2_rs2470890	0.76	0.13	0.42	0.014[d]	0.75	0.34
CYP1A2_rs762551	0.81	0.10	1.00	0.71	0.98	0.91
CYP2C9_rs1057910[e]	—	0.13	0.05	—	—	—
ACSL5_rs17129748	0.10	0.11	0.63	0.15	0.91	0.19
ACSL5_rs2419629	0.19	0.45	0.99	0.20	0.43	0.29
ACSL5_rs7919710	0.12	0.27	0.91	0.19	0.86	0.20
PPARG_rs1801282	**0.0059**[d]	0.36	0.37	**0.023**[b]	0.13	0.11
PPARG_rs3856806	0.033[c]	0.27	0.95	0.23	0.020	0.37
PTGS2_rs5275	0.37	0.54	0.78	0.77	0.63	0.84

Nominal *P*-value, $P < 0.05$; Bonferroni correction $P < 0.0017$. Bold values indicate the most significant associations.
[a]*P*-values for log-additive model are shown (except when indicated).
[b]*P*-value for dominant model.
[c]*P*-value for codominant model.
[d]*P*-value for recessive model.
[e]Genotyping rate under 50%.
[f]Adjusted by energy intake.

epidemiological interviews and genetic analysis. Serum lipid levels 1 year prior to CRC diagnosis were available only for 272 cases and 625 controls. A description of the subjects' characteristics is in Table I. Cases had larger reported weight and BMI 1 year before the diagnosis. Also had more often family history of CRC, lower physical activity and vegetables intake. Long-term regular use of aspirin and statins were also inversely associated with CRC risk.

Though energy intake was similar for cases and controls, total fat intake and the proportion of energy derived from fat (nutrient density) was larger for cases than controls. Serum lipid levels [high-density lipoprotein (HDL) and low-density lipoprotein (LDL)] measured 1 year prior to diagnosis were similar in cases and controls.

Fatty acid SNPs and CRC risk

Thirty SNPs in eight fatty acid biosynthesis and metabolism genes were genotyped in CRC cases and controls. A detailed description of the allele and genotype frequencies for the studied SNPs is in supplementary Table I (available at *Mutagenesis* Online). Two SNPs (rs16940302 of *LIPC* and rs1057910 of *CYP2C9*) were not in Hardy–Weinberg equilibrium. Nevertheless, none of these two SNPs showed significant associations with the variables included in this study, CRC risk or serum lipid levels. The *P*-values for main-effect associations assessed between the SNPs and CRC risk, serum lipid levels, BMI and dietary fat intake are summarized in Table II. Except for those indicated in the table, the reported *P*-values correspond to the log-additive model and are adjusted for age and sex.

We first considered whether genetic variation in these genes was associated with CRC risk. An association between selected SNPs in *LIPC* gene and CRC risk was observed. These associations remained statistically significant when BMI, serum lipid levels (HDL and LDL) and dietary fat intake were also included in the model as potential confounders. Weak associations were also observed between polymorphisms in *PPARG* gene and CRC risk. Details for the analysis of these SNPs are shown in Table III. The association for *LIPC* rs9652472 with CRC risk was significant after Bonferroni correction. The G allele of this SNP was associated with a per-allele OR of 1.52 (95% CI 1.20–1.92, *P*-value = 0.0005).

Since multiple SNPs were assessed for *LIPC* and three of them showed significant associations, a haplotype analysis was performed. However, this was not more informative than the analysis of individual SNPs since they were chosen as haplotype tagging SNPs. This large gene contains four haplotype blocks (supplementary Figure 1, available at *Mutagenesis* Online). The frequencies were under 0.15 and no associations between *LIPC* haplotypes and CRC risk were observed (data not shown).

Fatty acid SNPs and serum lipid levels

Serum lipid levels 1 year before diagnosis were only available for a limited number of subjects (272 cases and 625 controls).

Thus, the power to detect associations with SNPs was reduced. Only the SNP rs1800588 in *LIPC* was marginally associated to HDL levels in MECC study participants (Table IV). The T allele of this SNP was associated with higher HDL levels; mean differences were increased in 1.61 points per allele (95% CI 0.20–3.01, *P*-value = 0.025). No SNP was related to LDL serum levels.

Fatty acid SNPs and BMI

Selected polymorphism in the *LIPC* gene, specifically the SNPs rs16940302, rs7174210, rs4775053, and rs4775072, were found to be associated with changes in the median of BMI of the MECC study participants. Moreover, an association between rs2470890 in *CYP1A2* gene and rs1801282 in *PPARG* gene and BMI were also observed (Table II, for details see Table V). None of these associations remained statistically significant after Bonferroni correction.

Fatty acid SNPs and diet

The potential association of the SNPs with energy intake, fat intake and physical activity was also explored to exclude unexpected findings. None of the SNPs analysed was related to these behavioural variables (Table II).

Path analysis of SNPs, dietary fat intake, lipid levels, BMI, physical activity, energy and CRC

The main aim of this analysis was to perform a combined analysis of all players in the lipid metabolism. Path analysis using SEMs provide an interesting tool for this since a simultaneous estimate of associations can be done for an *a priori* proposed model. We built the model from the observed associations in the multiple univariate analyses previously described and tried to fit SEMs to identify the associations resistant to adjustment for indirect effects.

Figure 1 shows the proposed model reduced to relevant associations. The continuous arrows show the associations that remain significant in the SEM model. Discontinuous arrows show associations that were no longer significant and could be considered as indirect effects. This analysis confirms the direct effect of *LIPC* rs9652472 polymorphism on CRC and the indirect effect of several other *LIPC* polymorphisms through serum lipid levels. It is remarkable that some of these associations were weak and not significant in the univariate analysis, but the path analysis that accounts for global confounding has revealed the associations. Other behavioural variables like total energy intake, fat intake and physical exercise also remain related among each other and associated to colon cancer, showing the relevance of these factors as mediator variables. SNPs of *PPARG* in the path analysis lose the direct effect on CRC risk, but rs1801282 maintains its association to BMI. Supplementary Table II (available at *Mutagenesis* Online) shows the coefficients and *P*-values with the final model of the path analysis.

Table III. Association between polymorphisms in fatty acid metabolism genes and CRC risk

SNP	Controls, *N* (%)	Cases, *N* (%)	OR[a]	95% CI	*P*-value
LIPC_rs16940372					0.012
A/A	1029 (69.7)	621 (73.2)	1.00		
A/G	403 (27.3)	215 (25.4)	0.84	0.68–1.04	
G/G	45 (3)	12 (1.4)	0.41	0.20–0.84	
Log-additive	1477 (63.5)	848 (36.5)	0.65	0.64–0.94	0.0086
LIPC_rs4775053					0.021
C/C	1184 (80.1)	667 (82.3)	1.00		
T/C	277 (18.7)	138 (17.0)	0.78	0.61–1.00	
T/T	18 (1.2)	5 (0.6)	0.32	0.09–1.09	
Log-additive	1479 (64.6)	810 (35.4)	0.74	0.59–0.93	0.0099
LIPC_rs4775072					0.011
C/C	1380 (93.1)	768 (90.1)	1.00		
T/C	100 (6.7)	87 (9.6)	1.66	1.18–2.32	
T/T	2 (0.1)	2 (0.2)	2.10	0.29–15.22	
Log-additive	1482 (63.5)	852 (36.5)	1.64	1.19–2.25	0.0028
LIPC_rs9652472					0.0018
A/A	1269 (86.2)	694 (81.8)	1.00		
A/G	195 (13.2)	150 (17.7)	1.57	1.22–2.02	
G/G	8 (0.5)	4 (0.5)	1.43	0.40–5.15	
Log-additive	1472 (63.4)	848 (36.6)	1.52	1.20–1.92	0.0005
PPARG_rs1801282					0.017
C/C	1307 (88.4)	710 (87.4)	1.00		
C/G	163 (11.0)	102 (12.6)	1.11	0.84–1.48	
G/G	9 (0.6)	0 (0.0)	0.00	—	
Log-additive	1479 (64.6)	812 (35.4)	0.99	0.75–1.29	0.92
PPARG_rs3856806					0.033
C/C	1285 (86.8)	682 (83.9)	1.00		
T/C	182 (12.3)	128 (15.7)	1.31	1.01–1.70	
T/T	13 (0.9)	3 (0.4)	0.34	0.07–1.51	
Log-additive	1480 (64.5)	813 (35.5)	1.16	0.91–1.48	0.22

[a]Age, sex, BMI 1 year before diagnosis, serum lipid levels and total dietary fat intake adjusted OR. Only significative associations are shown.

Discussion

We have examined the association between polymorphisms in fatty acid metabolism genes and the risk of CRC in the context of energy balance accounting for behavioural variables: dietary intake and physical exercise and mediator variables: BMI and serum lipid levels. Among the genes studied, *LIPC* and *PPARG* have shown a significant association to CRC.

We have focused this analysis in fatty acid metabolism as an extension of our previous study on cholesterol metabolism (18). Though both pathways could be interrelated, the postulated carcinogenic mechanism for fatty acids would be more related to energy balance and possibly to insulin that has not been studied in the present study.

LIPC, serum lipids and CRC risk

The *LIPC* gene encodes a hepatic triglyceride lipase, which is highly expressed in the liver, and has the dual function of triglyceride, phospholipids and lipoproteins hydrolase and binding factor for receptor-mediated lipoprotein uptake (24–26). This gene has been extensively studied in relation to cardiovascular diseases (27–36). Hyperlipidaemia, characterized by increased total cholesterol (TC), triglycerides, and LDL and decreased HDL, has also been associated with cancer risk (37). Both genetic and lifestyle factors (such as

Table IV. Association between polymorphisms in fatty acid metabolism genes and serum lipid levels 1 year before CRC diagnosis.

SNP	*N*	Mean	SE	Difference[a]	95% CI	*P*-value
HDL						
LIPC_rs1800588						0.057
C/C	555	49.85	0.53	0.00		
T/C	243	50.68	0.71	1.17	−0.56 to 2.89	
T/T	31	55.35	2.29	4.54	0.39 to 8.68	
Log-additive	829			1.61	0.20 to 3.01	0.025

[a]Age, sex, BMI 1 year before diagnosis and total dietary fat intake adjusted OR. Only significative associations are shown.

Table V. Association between polymorphisms in fatty acid metabolism genes and BMI

SNP	*N*	Mean	SE	Difference[a]	95% CI	*P*-value
LIPC_rs4775072						0.074
C/C	1959	27.12	0.11	0.00		
T/C	162	27.98	0.43	0.84	0.08 to 1.60	
T/T	4	29.02	0.56	1.61	−2.89 to 6.12	
Log-additive	2125			0.84	0.12 to 1.56	0.023
LIPC_rs7174210						0.032
G/G	1704	27.06	0.11	0.00		
T/G	391	27.71	0.27	0.68	0.16 to 1.19	
T/T	25	26.69	0.95	−0.40	−2.50 to 1.45	
Log-additive	2120			0.48	0.02 to 0.95	0.042
CYP1A2_rs2470890						0.036
T/T	598	27.20	0.20	0.00		
T/C	1079	27.36	0.15	0.18	−0.28 to 0.65	
C/C	431	26.73	0.23	−0.51	−1.09 to 0.07	
Log-additive	2108			−0.22	−0.50 to 0.07	0.15
PPARG_rs1801282						0.066
C/C	1871	27.10	0.11	0.00		
C/G	243	27.86	0.31	0.74	0.12 to 1.37	
G/G	9	27.20	0.15	−0.03	−3.03 to 2.98	
Log-additive	2123			0.64	0.05 to 1.22	0.032

[a]Age, sex, serum HDL and LDL levels and total dietary fat intake adjusted OR. Only significative associations are shown.

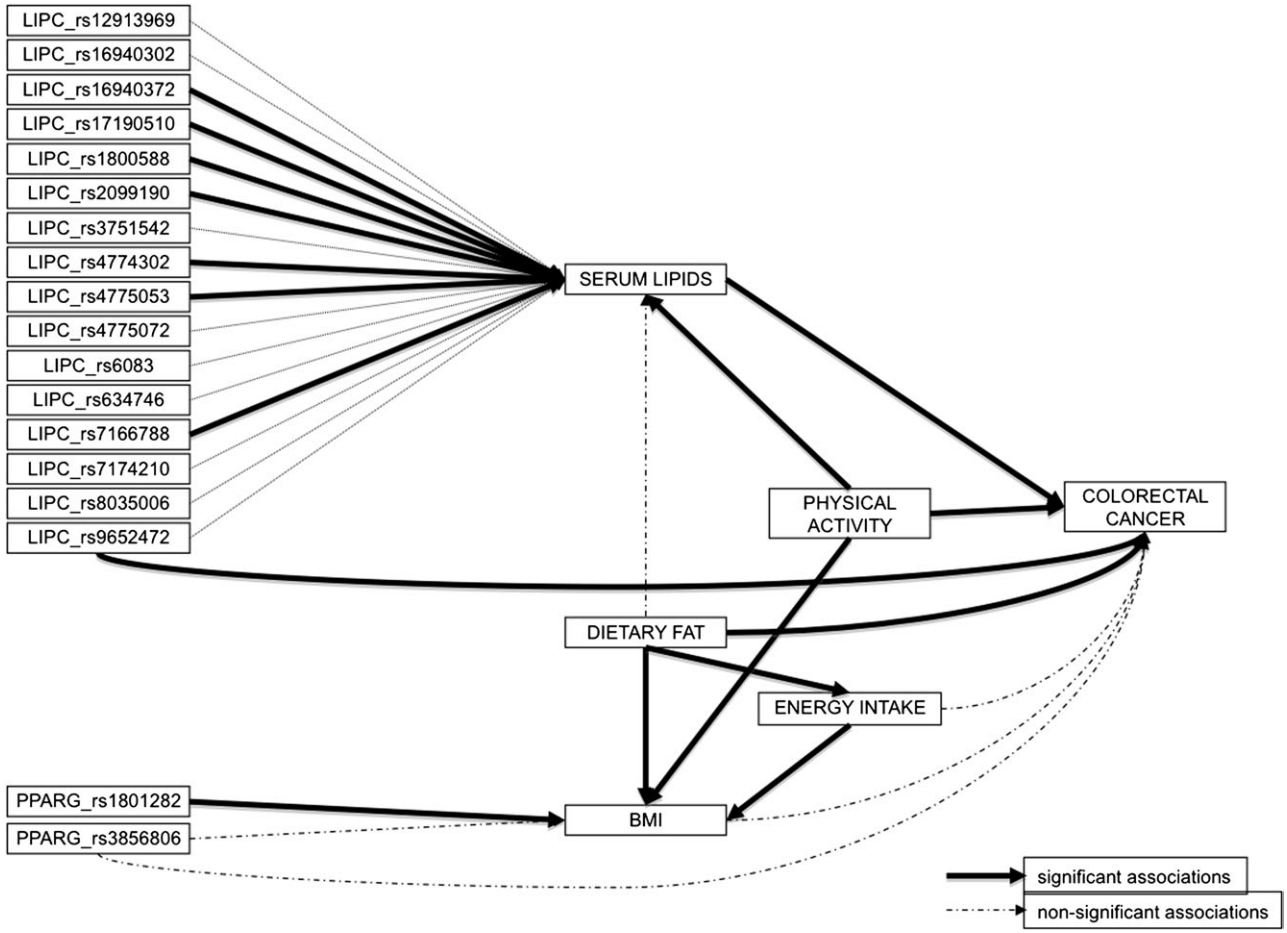

Fig. 1. Path analysis using SEM in R. The continuous arrows show the associations that remain significant in the SEM model ($P > 0.02$). Discontinuous arrows show associations that were no longer significant and could be considered indirect effects.

diet or body weight) affect serum lipid levels. Hyperlipidaemia has been associated with several genetic polymorphisms in the lipid metabolism pathway (28–31,38). Thus, the potential role of the *LIPC* gene in fatty acid metabolism is of particular interest in colorectal carcinogenesis, possibly mediated through hyperlipidaemia.

In this study, several polymorphisms in *LIPC* have been found to be associated with CRC risk, although no particular haplotype

was related to CRC. The most prominent was the association of the G allele of *LIPC* rs9652472 with increased risk of CRC. This effect is significant after Bonferroni correction and remains in a SEM that includes all the observed significant associations among intervening variables.

We also replicate the already described associations between the T allele of the *LIPC* SNP rs1800588 and higher serum HDL levels (28,29,31). It is assumed that this association is due to the change of the rate of transcription of the *LIPC* gene for the carriers of the T allele of this promoter polymorphism (32,39,40). Other studies also have reported that the T allele is associated with higher TC and triglycerides (41).

It is interesting that the *LIPC* SNP most associated to CRC, rs9652472, is different than that associated to HDL levels. Though there is a degree of linkage disequilibrium between both SNPs, the *LIPC* gene is large and complex, with 14 exons that have evolved in different haplotype blocks (supplementary Figure 1, available at *Mutagenesis* Online). Our results showed a direct effect of *LIPC* gene polymorphisms on colorectal carcinogenesis and also an indirect effect mediated through serum lipid levels, even including dietary fat intake in the models as potential confounders. This leaves open the possibility that this gene has an effect on CRC by an additional mechanism independent of lipid metabolism.

Two previous studies have analysed the association between polymorphisms in the hepatic lipase gene (*LIPC*) and CRC risk, but with conflicting results. While the study by Webb *et al*. (42) reported an association between 10 polymorphisms and CRC risk, including the SNP rs3829462 in the *LIPC* gene, Frank *et al*. (43) did not replicate these associations. The SNP most associated in our study, rs9652472, is in linkage disequilibrium with rs3829462 (D' = 1), though due to the low allele frequency of both SNPs (below 3% in CEU population) the concordance of alleles is not high ($r^2 = 0.001$).

Additional evidence on the role of polymorphisms in lipid metabolism genes in CRC is scant. The recent study from Hoeft *et al*. (44), after the examination of 43 fatty acid metabolism-related genes (from 392 tagged SNPs) in 1225 CRC cases and 2032 controls of the EPIC cohort, reported associations between polymorphisms in *HPGD*, *PLA2G6*, *TRPV3* and *PTGER2* genes and the risk of CRC. This study selected a different set of genes than ours (only one SNP in *PPARG* was coincident and *LIPC* was not analysed at all) and was focused exclusively on main genetic effects, ignoring other mediator variables as we have analysed.

PPARG, BMI and CRC risk

The *PPARG* gene encodes the gamma subunit of the peroxisome proliferator-activated receptor subfamily of nuclear receptors. This protein plays an important role in the regulation of adipocyte differentiation, glucose and lipid homeostasis, and intracellular insulin-signalling events. Consequently, *PPARG* has been implicated in the pathology of numerous diseases including obesity, diabetes, atherosclerosis and cancer (45–52). Moreover, experimental evidence has suggested that activation of *PPARG* in the colon results in growth inhibition and differentiation and reduces the malignant potential of CRC cells (53,54).

We observed that the T allele of the *PPARG* SNP rs1801282, which results in a proline-to-alanine substitution in codon 12 with a clear functional effect, was weakly associated with a CRC risk reduction. This is in accordance with other studies (14,51,52,54–58).

The path analysis has revealed that the effect of *PPARG* gene polymorphisms on colorectal carcinogenesis is mediated through BMI. The univariate analysis showed significant direct associations between *PPARG* and CRC, but the model that considered other mediators such as dietary fat intake, energy and physical activity only retained as significant the association between *PPARG* rs1801282 and BMI.

Strengths and limitations

This study is one of the largest conducted so far in which polymorphisms in lipid metabolism have been studied in the global context of diet, physical activity and serum lipid levels in relation to CRC risk. The population-based case-control design, though restrospective, is capable to identify a series of associations that allow a global picture of the key players. Some of the reported associations had been previously reported in studies of specific factors, which reinforces the strength of the proposed model.

Probably the study's most important limitation is that the data on blood lipids were only available for lipids related with cholesterol (HDL, LDL and TC) and in a limited subset of the study. The estimates for the associations with these variables were less precise, but could be incorporated into the comprehensive path analysis since this statistical technique only requires the matrix of correlation coefficients. The analyses performed in this study only used HDL and LDL, which predominantly reflect the cholesterol metabolism rather that fatty acid metabolism which we were interested in. The availability of serum triglyceride would have been interesting, but this is not routinely requested in blood tests and could not be retrieved. Anyway this is, as far as we know, the only study on CRC that explores simultaneously the effects of polymorphisms in lipid metabolism genes with diet, BMI and blood lipid levels.

The sample size of this study, though large, is not powered enough to assess gene–gene or gene–environment interactions. The focus of our study has been the main effects, but further investigations should be done in larger or pool studies on the putative role of gene–gene interactions in this pathway.

Conclusion

Although our findings requires validation in other independent populations, further characterization of *LIPC* and *PPARG* polymorphisms may provide new insights into their contribution to incidence and progression of CRC.

Supplementary data

Supplementary Figure 1 and Table 1 are available at *Mutagenesis* Online.

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