COLORECTAL CANCER

The C/C$_{-13910}$ genotype of adult-type hypolactasia is associated with an increased risk of colorectal cancer in the Finnish population

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Background and aims: The role of nutrition in the pathogenesis of colorectal cancer is not fully understood. Milk products are an essential part of human nutrition in Western countries. Absorption of lactose, the main sugar of milk, is regulated by the activity of the lactase enzyme in the gut wall. The activity of lactase is genetically determined and is associated with a C/T single nucleotide polymorphism residing 13910 bp upstream of the lactase coding sequence. Here we have studied the relationship between the C/T$_{-13910}$ polymorphism and colorectal cancer in Finnish, British, and Spanish populations.

Patients and methods: A total of 2766 subjects, including 963 Finnish, 283 British, and 163 Spanish subjects with colorectal cancer, and 773 Finnish, 363 British, and 221 Spanish control subjects, were genotyped for the C/T$_{-13910}$ variant by polymerase chain reaction minisequencing.

Results: The C/C$_{-13910}$ genotype, which is a robust molecular marker of low lactase activity (lactase non-persistence), was found to significantly associate with the risk of colorectal cancer ($p = 0.015$) in the Finnish subjects, with an odds ratio of 1.40 (95% confidence interval 1.07–1.85). No association was found with site, histology, or stage of the tumour. No significant risk was detected in the British or Spanish populations.

Conclusion: Low lactase enzyme activity, defined by genotyping of the C/T$_{-13910}$ variant, may increase the risk of colorectal cancer. Further studies are warranted to investigate the role of milk and other dairy products in the pathogenesis of colon cancer in different populations.

Both genetic and environmental factors are associated with colorectal cancer; the second most common cause of cancer death in the Western world.[1] Nutrition is put forward as an epigenetic modulator in the pathogenesis of colorectal cancer. Milk and other dairy products are important components of the Western diet. Lactose, the main sugar of milk, is hydrolysed to glucose and galactose by the lactase enzyme in the intestinal wall. Absorption of lactose depends on the genetically determined activity of lactase. Downregulation of lactase activity occurs after weaning in half of the world's population, making them unable to digest lactose and causing abdominal symptoms such as bloating, diarrhoea, and flatulence.[2]

The role of dairy products as a risk factor for colorectal cancer has remained controversial.[3–10] A modest reduction in the risk of colorectal cancer and the consumption of dairy products has been suggested in several cohort studies[3–5] whereas in case control studies a protective association has not been demonstrated in the main.[4 9] The protective effect of dairy products have been proposed to be mediated by several different components of milk, including calcium,[4–10] vitamin D,[4 6–10] lactose,[3] lactoferrin,[11] and conjugated linoleic acid.[12]

Studies on the effect of lactase activity on various pathological conditions have been based on indirect tests (the lactose tolerance test and the breath hydrogen test) with varying specificity and sensitivity.[13] Recently, a single nucleotide polymorphism, C/T$_{-13910}$, residing 13910 bp upstream of the lactase encoding gene lactase-phlorizin hydrolase (LPH), has been shown to strictly associate with lactase persistence.[14 15] The C/C$_{-13910}$ genotype defines lactose malabsorbers (lactase activity <10 U/g protein), and genotypes C/T$_{-13910}$ and T/T$_{-13910}$ lactose absorbers (lactase activity ≥ 10 U/g protein). These three genotypes are perfectly related with the level of lactase activity in intestinal biopsy samples and their lactase/sucrase ratio and with the results of the indirect lactose tolerance test.[14–17] Furthermore, molecular epidemiological studies have shown that the prevalence of the C/C$_{-13910}$ genotype is consistent with previously published values for adult-type hypolactasia in several populations (for example, French and Utah populations).[14] Functional evidence for the C/T$_{-13910}$ variant in the regulation of lactase activity has been obtained in several studies.[15 18 19] Individuals with the persistent T$_{-13910}$ allele show several times higher levels of LPH mRNA in their intestinal mucosa compared with that found in individuals with the non-persistent C$_{-13910}$ allele, suggesting regulation of the LPH gene at the transcriptional level.[15] This is in agreement with recent in vitro studies reporting differential transcriptional regulation of lactase promoter by the C and T variants; a greater increase in lactase promoter activity shown by the T$_{-13910}$ variant.[18 19]

This study addresses the question of whether there exists an association between lactase activity and colorectal cancer in three populations (Finnish, British, and Spanish patients and their corresponding controls) by genotyping the C/T$_{-13910}$ variant.

Abbreviations: LPH, lactase-phlorizin hydrolase; PCR, polymerase chain reaction; OR, odds ratio; LM, lactose malabsorption

Table 1 Participants in the study

Group	Clinical data	Cases (n)	Controls (n)
Finland (A)	+	773	773
Finland (B) (West)	−	79	−
Finland (C) (East)	+	111	−
UK	−	283	363
Spain	−	163	221
Total		1409	1357

SUBJECTS AND METHODS

To assess the role of lactase activity as a risk factor for colorectal cancer in the Finnish population, we genotyped a total of 963 Finnish subjects with colorectal cancer and 773 controls for the C/T_{-13910} variant by polymerase chain reaction (PCR) minisequencing (table 1). Patients in group A (n = 773, males/females 400/373, median age 69 years (range 23–93)) were treated at nine large regional hospitals in Finland. Due to wide regional variation in the prevalence of the C/C_{-13910} genotype in Finland,[14] the control group (n = 773) was frequency matched to cases and derived from the same geographical province of Finland. Control subjects were participants in a Finnish multicentre study, FinnDiane, aiming to elucidate the genetic background of diabetic nephropathy, and they had been genotyped for the C/T_{-13910} variant associated with lactose malabsorption (LM).[20] To increase the number of healthy controls, we genotyped an additional 34 Finnish blood donors from the corresponding geographical areas. All samples were handled anonymously.

Moreover, two sample sets of colorectal cancer patients from the Western and Eastern parts of Finland were genotyped for the C/T_{-13910} variant (groups B and C). The first group of patients (B) (n = 79) who originated from the Western coast of Finland were operated on and treated during the year 2001 at the central hospital of Vaasa. Anonymous paraffin embedded tissue blocks were obtained from these patients. The second group (C) (n = 111, males/females 59/52, median age 70 years (range 29–87)) of patients originated from the East, the old Karelian part of Finland, a province which today belongs to Russia. No frequency matched control subjects were available for these two subgroups of Western and Eastern patients (table 1).

To extend the study to other populations, samples from 283 colorectal cancer patients and 363 healthy controls from the UK and from 163 colorectal cancer patients and 221 control subjects over 65 years old from the Hospital Universitari Vall d'Hebron at Barcelona, Spain, were analysed. Cases from UK were affected by colorectal cancer before the age of 75 years and were primarily derived from southern England. Controls were derived from the same geographical area and none was affected by gastrointestinal malignancy. All of the Spanish subjects were residents in Catalonia. Samples were handled anonymously in accordance with previously established ethical protocols. The study was approved by the ethics committee of the Helsinki University Central Hospital and the corresponding ethics committees of the other institutions involved. All subjects gave their written informed consent.

Clinical data

Clinical data, including age at the time of diagnosis, sex, tumour histology, stage, and site of the tumour were obtained from 884 Finnish patients (groups A and C). Tumours were classified as adenocarcinomas, mucinous adenocarcinomas, or as partly mucinous adenocarcinomas, and also according to stage and location. The site of the tumour was divided into six groups: caecum, ascending colon, hepatic flexure and transverse colon, splenic flexure and descending colon, sigmoid, and rectum. The clinical diagnosis of colorectal cancer in the Finnish patients was made at a mean age of 67.6 (11.9) years. Clinical parameters of these cases are depicted in table 2. No clinical details were available from a small subgroup of Finnish patients and the British and Spanish patients.

Genotyping

DNA of the study subjects was extracted using standard protocols. The DNA fragment spanning the C/T_{-13910} variant was amplified using one biotinylated (5′-CCT CGT TAA TAC CCA CTG ACC TA-3′) primer and one unbiotinylated (5′-GTC ACT TTG ATA TGA TGA GAG CA-3′) primer. PCR amplifications were carried out in a 50 µl volume with genomic DNA (100 ng), primers (20 ng each), dNTPs (200 µM), and 0.5 U of Taq polymerase in a standard buffer (Dynazyme; Finnzymes, Espoo, Finland). PCR cycle conditions were as

Table 2 Clinical characteristics of the Finnish colorectal cancer patients*

	C/C (n (%))	C/T and T/T (n (%))	Total	p Value
Age at diagnosis (y)	67.4 (11.8)	67.6 (11.9)		
Sex				
Male	114 (54.0%)	341 (51.0%)	455 (51.7%)	NS
Female	97 (46.0%)	328 (49.0%)	425 (48.3%)	
Tumour site				
Caecum	26 (12.4%)	79 (11.8%)	105 (12.0%)	NS
Ascending colon	19 (9.1%)	74 (11.1%)	93 (10.6%)	
Hepatic flexure and transverse colon	21 (10.0%)	59 (8.9%)	80 (9.1%)	
Splenic flexure and descending colon	16 (7.6%)	39 (5.9%)	55 (6.3%)	
Sigmoid	42 (20.0%)	128 (19.2%)	170 (19.4%)	
Rectum	86 (41.0%)	288 (43.2%)	374 (42.6%)	
Tumour stage				
I	48 (22.9%)	130 (19.6%)	178 (20.3%)	NS
II	76 (36.2%)	270 (40.6%)	346 (39.5%)	
III	57 (27.1%)	182 (27.4%)	239 (27.3%)	
IV	29 (13.8%)	83 (12.5%)	112 (12.8%)	
Tumour histology				
Adenocarcinoma	200 (94.8%)	607 (90.7%)	807 (91.7%)	NS
Mucinous adenocarcinoma	7 (3.3%)	35 (5.2%)	42 (4.8%)	
Partly mucinous adenocarcinoma	4 (1.9%)	27 (4.0%)	31 (3.5%)	

*Data for various clinical characteristics were not available from all subjects.

follows: an initial round of denaturation at 94°C, then 35 cycles at 94°C for 30 seconds, 53°C for 30 seconds, 72°C for 75 seconds, and a final extension of 72°C for 10 minutes. The PCR product (10 µl) was captured in a streptavidin coated microtitre well (Thermo Electron, Helsinki, Finland); two parallel minisequencing reactions were carried out for each PCR product. The wells were washed and bound DNA denatured. The minisequencing reaction contained 10 pmol of the minisequencing primer for C/T_{-13910} (5′-GGC AAT ACA GAT AAG ATA ATG TAG-3′) and 0.1 µl of either H-dCTP or H-dTTP (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK), and 0.05 U of DNA polymerase (Dynazyme II, Finnzymes, Espoo, Finland). The microtitre wells were incubated for 15 minutes at 56°C and washed. The detection primer was eluted, and radioactivity measured in a liquid scintillation counter (Rackbeta 1209; Wallac, Turku, Finland).[21]

Statistical analyses

The relationship between lactase genotypes and risk of colorectal cancer was assessed by means of odds ratios (ORs) with 95% confidence limits (CI) calculated by unconditional logistic regression. Studies were weighted according to the inverse of the standard error of the log of the OR. To test for population stratification, the distribution of genotypes in cases and controls was tested for a departure from Hardy-Weinberg equilibrium by means of the χ^2 test. Frequency differences were analysed with Pearson's χ^2 test. A p value less than 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS for Windows, release 11.5.1 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

The lactase genotype distributions in the colorectal cancer patients and controls are shown in table 3. Interestingly, the C/C_{-13910} genotype was found more often in cancer patients than in controls in the group of Finnish subjects ($p = 0.015$). The OR for having genotype C/C_{-13910}, associated with low lactase activity, versus C/T_{-13910} or T/T_{-13910}, associated with high lactase activity and colorectal cancer, was 1.40 (95% CI 1.07–1.85). In British subjects, there was no difference in the genotype distribution of cases and controls (OR 1.05 (95% CI 0.59–1.86)), and similarly in the Spanish subjects no association was observed (OR 0.81 (95% CI 0.59–1.10)). OR for the three populations combined was 1.07 (95% CI 0.89–1.29); $p = 0.48$). Allelic frequencies were tested for Hardy-Weinberg equilibrium and they did not significantly diverge from it in any of the three populations.

There was no difference between the mean age of Finnish patients with the C/C_{-13910} genotype (mean age 67.4 (11.8) years) and patients with non-C/C genotypes (mean age 67.6 (11.9) years). When comparing the clinical characteristics of the patients, no significant differences for site, stage, or histology of the tumour for C/C_{-13910} or non-C/C_{-13910} genotypes were detected (table 2). Also, there were no differences in the distribution of the C/C_{-13910} genotype among male and female patients.

The overall prevalence of the C/C_{-13910} genotype in Finnish subjects (patients and controls; n = 1736) was 20.6%, which is in accordance with the frequency of 18.1% found in the original study in the Finnish population.[14] The prevalence of the C/C_{-13910} genotype in patients originating from the Swedish speaking region in Western Finland (n = 79) was 10.1%. The frequency of LM has been shown to be lowest among Swedish speaking population of Finland. The frequency of 10.1% is in agreement with the frequency of 8.7% in an earlier study of the prevalence of the C/C_{-13910} genotype and LM in the Swedish speaking population of Finland.[20] The prevalence of LM among patients born in Karelia (n = 111) was 26.1%, which is in line with our original study showing an average LM prevalence of 23% in Eastern Finland.[14]

The frequency of LM is strongly dependent on ethnic origin. The overall frequency of the C/C_{-13910} genotype in British subjects (patients and controls; n = 646) was 9.0%. This value is higher than the earlier reported frequency of 5% of LM in the UK,[22 23] possibly reflecting recent immigration and mixing of the current population in Southern England. The overall frequency of the C/C_{-13910} genotype in Spanish subjects (patients and controls; n = 384) was 34.6%. The study subjects represent a sample of residents in Catalonia, a region from which, to our knowledge, the frequency of LM has not been published previously. However, the frequency was higher than the earlier reported values for adult-type hypolactasia in Spain, which ranged from 19% in Southern Spain to 28% in Valencia.[24 25]

DISCUSSION

Here, we have demonstrated that the genotype C/C_{-13910}, defining low lactase activity in the intestinal wall, was associated with an elevated risk of colorectal cancer in the Finnish population. However, no such association was detected in the British and Spanish populations. There was some correlation between the risk value and average milk consumption among the populations, the Finns consuming the highest amount of milk products (table 3).[26] If consumption of yoghurt and cheese is added, the difference in dairy product consumption is even greater between Finland and Spain and between Finland and the UK.[26] Unfortunately, data were missing for consumption of milk products in the study materials.

It is noteworthy that the incidence of colorectal cancer in Finland is among the lowest in the developed countries.[27] Furthermore, compared with high incidence areas of colorectal cancer, Finns have a higher intake of milk and dietary fibre. Our results may reflect the protective effect of milk in lactose absorbers able to use milk products or be a consequence of an effect of some other component(s) of milk. Alternatively, our findings raise the question of

Table 3 C/T_{-13910} genotype distribution in Finnish, British, and Spanish subjects

		C/C_{-13910}		C/T_{-13910}		T/T_{-13910}		Total			Milk consumption/ person (kg/year)†
		n	%	n	%	n	%	n	OR (95% CI)	p Value*	
Finland	Cases	182	23.5	383	49.5	208	26.9	773	1.40 (1.07–1.85)	0.015	186
	Controls	139	18.0	392	50.7	242	31.3	773			
UK	Cases	26	9.2	100	35.3	157	55.5	283	1.05 (0.59–1.86)	NS	113
	Controls	32	8.8	139	38.3	192	52.9	363			
Spain	Cases	52	31.9	77	47.2	34	20.9	163	0.81 (0.59–1.10)	NS	123
	Controls	81	36.7	116	52.5	24	10.9	221			

*Genotype C/C_{-13910} versus C/T_{-13910} and T/T_{-13910}.
†The World Dairy Situation 2002, Bulletin of the International Dairy Federation 378/2002.

whether the risk depends on the amount of milk (and lactose) consumed by lactose malabsorbers? It is intriguing that in adulthood, 11–32% of lactose malabsorbers report no symptoms from lactose containing milk products.[28 29] However, there is evidence that subjects with lactose malabsorption use less milk products than absorbers.[30] This is supported by a prospective Finnish cohort study in which the consumption of milk and lactose was suggested to have an inverse effect on colon cancer, with a multivariate adjusted relative risk of 0.31 in the highest versus the lowest quartile of lactose intake.[3]

Little is known of the changes in bacterial flora and their role in colorectal carcinogenesis. In lactose malabsorption, the ingested lactose is not digested in the small intestine, thereby allowing most of the lactose to enter the colon and to be rapidly metabolised by colonic bacteria.[31] One of the main products of fermentation is butyrate, a major energy source for colonocytes. The role of butyrate as a control factor of cell proliferation has been established and is believed to be of importance in the prevention of colorectal cancer.[32] Ingestion of lactose causes alterations in the populations of intestinal microflora and their metabolism by reducing the pH in the colon and decreasing the aerobic flora.[31 33] Lactose may even promote the maintenance of several species of lactic acid producing bacteria. This is consistent with the observations of differences between the faecal floras of low and high risk populations of colon cancer and that some lactobacillus species and *Eubacterium aerofaciens*, which also produce lactic acid, show the closest associations with low risk of cancer.[34 35] Probiotics may improve lactose absorption.[36] In animal models of colorectal cancer, treatment with probiotics has been shown to reduce the prevalence of the disease. However, information on the state of adaptation of the colonic microflora to lactase activity in the intestine is lacking. The interaction of dietary components such as lactose with endogenous factors, including the microflora, is one of the major interests in current studies on colorectal cancer.

Furthermore, it has been proposed that galactose, a constituent of lactose, has a protective effect against colorectal cancer. In colon cancer, increased mucosal expression of terminal unsubstituted galactose has been detected, and this allows for interaction with mitogenic galactose binding lectins of dietary or microbial origin. It has been shown that lectins stimulate colon epithelial proliferation.[37] Lectin binding to human colonocytes is a predictive factor for the presence of colorectal malignant lesions.[38] It has been shown that the galactose content of dietary non-starch polysaccharide (fibre) show an overall significant protective effect for right sided colon cancer by inhibiting galactose binding lectins and preventing their interaction with the colonic epithelium.[37] Interestingly, a correlation between the level of lactose binding lectin (M_r 31,000) and the stage of progression of colorectal carcinomas has also been shown.[39 40]

Downregulation of lactase activity after weaning is a normal phenomenon. In terms of evolution, milk may not be a nutritional need in adulthood; high lactase activity being necessary only in early childhood to facilitate breast feeding. Consequently, one can hypothesise that a lactose load after weaning may have harmful effects in those with low lactase enzyme activity and only those who consume little milk products or have inherited the mutation of lactase persistence might be able to avoid them. This hypothesis would explain the population based differences observed in our study; lactase deficiency being associated with colorectal cancer only in the Finnish population known to display a high intake of milk products.[26] On the other hand, it is possible that our finding reflects lower dairy product consumption in lactose malabsorbers and supports the earlier findings of the protective effects of milk and lactose for colon cancer in the Finnish population.[3 5] Further studies, including data on the milk consumption in the study subjects, are needed to confirm our findings.

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