RESEARCH REPORT

Food and nutrient intakes and K-*ras* mutations in exocrine pancreatic cancer

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Background: No studies have investigated the relation between K-*ras* mutations and dietary factors in exocrine pancreatic cancer (EPC), and fewer than 10 studies have done so in other neoplasms.
Patients and Methods: Incident cases of EPC were prospectively identified, and interviewed face-to-face during hospital admission. Food and nutrient intakes were measured with a food frequency questionnaire. Logistic regression was used to compare EPC cases (n = 107) with and without K-*ras* mutations (case-case study).
Results: K-*ras* mutations were more common among daily consumers of milk and other dairy products than among non-daily consumers: the odds ratio adjusted by total energy, age, sex, smoking, alcohol and coffee consumption (ORa) was 5.1 (95% CI 1.1 to 24.5, p = 0.040). For all dairy products, including butter, the ORa for the medium and upper tertiles of intake were 5.4 and 11.6, respectively (p for trend = 0.023). The ORa for regular coffee drinkers further adjusted by dairy consumption was 4.7 (95% CI 1.1 to 20.7, p = 0.043). K-*ras* mutated cases reported a lower intake of vitamin E (ORa = 0.2, p for trend = 0.036), polyunsaturated fats and omega 3 fatty acids (ORa = 0.2; p for trend $<$0.03).
Conclusions: Results support the hypothesis that in EPC exposure to specific dietary components or contaminants may influence the occurrence or persistence of K-*ras* mutations.

Although the aetiology of exocrine pancreatic cancer (EPC) is poorly understood, important pathogenic clues have emerged from epidemiological and genetic studies.[1–4] The classic risk factors include age, male sex, cigarette smoking, and possibly diabetes and chronic pancreatitis. The only firmly established and modifiable risk factor is smoking, but it explains only a fraction of the incidence.[1]

Diet may also play a role in modifying risk of pancreatic cancer. Some studies found intake of fresh fruits, vegetables, fibre, vitamins C and D, carotenes and folate to be protective, whereas high caloric intake, dairy products, eggs, fats, carbohydrates and cholesterol have been seen to increase risk. However, the consistency of findings, specific dietary components and mechanisms remain unclear.[5–9]

Molecular pathology and molecular epidemiology studies suggest that wild-type K-*ras* EPC arise though genetic pathways distinct from those that harbour a K-*ras* mutation.[1 2 10 11] These somatic (acquired) mutations are the most frequent oncogene alteration in human cancers, and a prime example of activation by point mutation.[11 12] Ras proteins are critical regulators of cell function, including growth, differentiation and apoptosis, with membrane localisation of the protein being a prerequisite for malignant transformation, which promotes tumour proliferation, survival and invasion.[4 12 13] K-*ras* point mutations at codon 12, an early event in pancreatic carcinogenesis, are found in 75%–90% of pancreatic cancers, a frequency not encountered in any other solid neoplasm.[10–13] As early as 1983, mutated *ras* oncogenes were identified in experimental tumours of rodents that were exposed to chemical or physical carcinogens.[12] Evidence that *ras* genes can be involved in the initiation of carcinogenesis was obtained in 1985 when it was shown that H-*ras* oncogenes that were activated in chemically induced mammary carcinomas of rats were activated by the type of mutation that is known to be caused by the initiating carcinogen.[12 14] Therefore, laboratory experiments have shown that *ras* genes are critical DNA targets for chemical carcinogens.[3 12] Molecular epidemiological studies have suggested that in pancreatic cancer coffee[15–18] and some environmental compounds[1–3 10 14 17] might be associated with K-*ras* mutations; however, clinical and environmental processes with the potential to influence the occurrence and persistence of K-*ras* mutations in human neoplasms are still poorly understood.[3]

Studies in animals and humans suggest that dietary factors regulate the development of neoplasms through their effects on K-*ras* mutations. In colorectal tumours K-*ras* mutations have been associated with intake of cruciferous vegetables, meat, coffee, carbohydrates, monounsaturated and other dietary fats, vitamins B6 and B12, calcium, animal proteins and dietary folate;[19–26] the associations are often weak and inconsistent.[1] A lung carcinogenesis study in mice showed that a diet supplemented with vitamin E decreased the frequency of K-*ras* mutations.[27]

Despite the firmly established importance of K-*ras* mutations in the aetiopathogenesis of EPC, and the potential role of dietary components in modifying risk, the relation between diet and K-*ras* mutations has so far not been studied in this disease, and seldom in other neoplasms. The objective of the present study was therefore to analyse the relation between food and nutrient intakes and the prevalence at diagnosis of mutations in codon 12 of the K-*ras* oncogene in patients with EPC.

Abbreviations: EPC, exocrine pancreatic cancer; MUFA, monounsaturated fat; PANKRAS II, Multicentre Prospective Study on the Role of K-*ras* and other Genetic Alterations in the Diagnosis, Prognosis and Aetiology of Pancreatic and Biliary Diseases; PUFA, polyunsaturated fat; RE, retinol equivalents (1 RE = 1 μg = 5 IU of vitamin A); SFFQ, semi-quantitative food frequency questionnaire

PATIENTS AND METHODS

Selection of patients

Methods of the PANKRAS II study have been described in detail.[10 14–16 28–30] Briefly, subject recruitment took place in 1992–5 at five general hospitals in the eastern Mediterranean part of Spain, where 185 incident cases of EPC were prospectively identified. All their diagnoses were reviewed by a panel of experts and by the study reference pathologists, blinded to the original diagnoses and to molecular results.

Mutations in codon 12 of the K-*ras* gene could be tested for 121 cases, of whom 94 (77.7%) had mutations, and 27 did not.[10 14–16] The present report is based on 107 EPC patients with known K-*ras* status, and with information about dietary habits and potential confounders. There were no significant differences between them and the remaining EPC cases with respect to sex, education, study site, tumour stage, duration of the interview, energy intake and consumption of coffee, tobacco and alcohol, except that the included subjects were slightly younger.[15] The mean age of the 107 cases was 64.2 years (range 36.8–88.6), 42.1% were female, 83 (77.6%) harboured a K-*ras* mutation and 24 did not.[14–16] There were no differences in the frequency of mutations according to age, sex, education and tumour stage at diagnosis. Wild-type cases had smoked slightly more and drank significantly less coffee than mutated cases.[15] The study design was approved by the ethics committees of the participating hospitals, and patients gave informed consent to be included in the study.

Patient interviews and information on diet

A structured form was used to collect clinicopathological information from medical records, including details on diagnostic procedures, laboratory results and follow-up care.[29 30] Over 88% of the patients were interviewed face-to-face by trained monitors during hospital stay, close to the time of diagnosis. The respondent was the patient himself in 96% of the cases and a relative alone in 4%. Interviews included questions about past clinical history, symptoms, occupation, diet, coffee, alcohol and tobacco consumption.[10 15 29 30] Information was thus obtained on dietary habits. Patients were asked about the frequency of consumption of food groups during the year before the first symptom of the current illness. For this purpose a brief food frequency questionnaire (bFFQ) was administered; it consisted of 14 selected indicator food groups: milk and other dairy products, excluding butter; butter; eggs; red meat, chicken and organs; fish and shellfish; raw vegetables; cooked vegetables; fruit; bread; potatoes; cereals and legumes; sausage and cured meats; lard; and vegetable oil. In the bFFQ the frequency of consumption was divided into four categories: rarely or never; various times/month; various times/week; and daily, with an additional category of ''don't know/remember''.[28 30] To assess the reliability of interviews, a sample of relatives was concurrently and separately interviewed about the patient's clinical history and dietary habits, and agreement between the two sets of responses was compared.[30]

To estimate energy and nutrient content values for the food groups assessed in the PANKRAS study, we used data from a validated 93-item semi-quantitative food frequency questionnaire (SFFQ) with nine response categories administered to participants (n = 1337) in a nutrition and health population-based survey carried out in an area with dietary habits comparable to areas included in the PANKRAS study.[31] Nutrient intakes were calculated by multiplying the frequency of use for each food by the nutrient composition for the portion size specified on the SFFQ, summing across all foods to obtain total intakes for each individual.[28] The SFFQ appeared adequate for ranking subjects according to intake of most food groups and nutrients.[28] Correlations between actual and simulated intakes exceeded 0.70 for 10 of the groups assessed. Estimated intakes for four of the 14 food groups (milk and other dairy products, fresh vegetables, bread, and fruit) were more moderate (0.43–0.56). Correlations exceeded 0.60 for most nutrients.[28]

Detection of K-*ras* mutations

Details of laboratory protocols have also been described elsewhere.[10 14 15] Briefly, mutations in codon 12 of the K-*ras* oncogene were studied using DNA extracted from paraffin-embedded tumour tissue. DNA was extracted and amplified in two steps by nested PCR; in the second amplification reaction, an artificial BstNI restriction endonuclease site was introduced to discriminate between wild-type and mutated K-*ras* codon 12 sequences. Products were analysed by acrylamide gel electrophoresis and ethidium bromide staining. This technique is able to detect one homozygous mutated cell in the presence of 10^2 normal cells. To characterise the nucleotide substitution in codon 12, all mutated samples were further analysed using an RFLP-based approach. Interpretation of digestion products electrophoresis was performed independently by three investigators. When discordant results were obtained, the analysis was repeated and results evaluated again.

Statistical analyses

In this case-case study[32] we compared the dietary habits of the 83 cases of EPC with a K-*ras* mutated tumour and of the 24 cases of EPC whose tumours did not harbour such mutations. In the case-case design the odds ratio is a measure of gene-environment interaction; in our study, specifically, the interaction between dietary components and somatic mutations in the K-*ras* (proto)oncogene.[14 15 32] Categories of frequency of consumption for food groups were grouped as low, medium and high as shown below. All nutrients intakes were adjusted for total energy intake by calculating the residuals from a linear regression with the log(e) of the nutrient modelled as the dependent variable and the log(e) of total energy intake as the independent variable.[33] Univariate statistics were computed as customary.[34–36] Student's t test or Mann–Whitney U test were used to analyse normally or non-normally distributed quantitative variables, respectively. In contingency tables, Fisher's exact test for homogeneity or independence was applied to assess the relation between two categorical variables. When a tendency was observed Mantel–Haenszel's χ^2 test for linear trend was used. To estimate the magnitude of the associations between food group or nutrient intakes and K-*ras* mutations multivariate-adjusted odds ratios and their corresponding 95% confidence intervals (CI) were calculated by unconditional logistic regression.[32 36] Categorical ordinal variables were analysed for a linear dose-response relation through the multivariate analogue of Mantel's extension test using the likelihood ratio test with one degree of freedom. When a tendency was not clearly apparent, the likelihood ratio test statistic was used to asses significance. The likelihood ratio test was also applied to explore the joint effect of two variables by including in the model the two main terms and the interaction term between the two variables.[34–36] The following potential confounders were included in the basic models: age (continuous), gender, total energy intake (kcal/day, continuous), coffee consumption (non-regular and regular drinkers), smoking (never-, former- and current-smokers), and alcohol consumption (non-drinkers and drinkers). Allowance for other possible confounding variables did not materially alter the estimates. The level of statistical significance was set at 0.05, and all tests are two-tailed. Statistical analyses were performed using SPSS, version 12.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Daily consumers of milk and other dairy products were over five times more likely to have a K-*ras* mutated tumour than non-daily consumers: the odds ratio adjusted by total energy, age and sex was 6.30 (95% CI 1.56 to 25.41). The OR further adjusted by smoking and alcohol consumption was 7.58 (95% CI 1.73 to 33.25). Patients in the upper category of butter consumption were over three times more likely to have a K-*ras* mutated tumour. For all dairy products, including butter, the ORs for the medium and high categories of intake were 7.43 and 19.68, respectively (p for trend = 0.002) (table 1).

No statistically significant associations were observed between the rest of food groups and K-*ras* mutations, except that the intake of meat and sausages combined was higher among cases with wild-type K-*ras* tumours. However, weaker associations were also apparent with vegetables and fruits combined (ORs for medium and high tertiles of intake were 0.44 and 0.28, respectively, p for trend = 0.083), fish (OR for high tertile = 0.36, 95% CI 0.06 to 2.09), and vegetable oil (ORs for medium and high tertiles: 0.57 and 0.24, respectively, p for trend = 0.182) (table 1).

Table 2 presents the mutually-adjusted relations of dairy products and coffee consumption with K-*ras* mutations. Even after adjusting by coffee consumption, daily consumers of milk and other dairy products were five times more likely to have a K-*ras* mutated EPC than non-daily consumers (OR = 5.10, p = 0.040). The ORs for the medium and high categories of all dairy products were over 5 and 11, respectively (p for trend = 0.023). Our previous report on coffee did not take diet into account;[15] now, after adjusting by milk and other dairy products, regular coffee drinkers were still over four times more likely to have a K-*ras* mutated tumour than non-regular coffee drinkers ($p<0.05$). Furthermore, the odds ratios increased with increasing number of coffee cups (p for trend = 0.034) (table 2). There was no evidence of an interaction between dairy products and coffee. After adjusting for calcium intake the estimates for dairy products were significantly increased, while for coffee they remained unchanged.

There were no significant differences between mutated and wild-type cases in total energy intake, or in the intakes of total proteins, total fats and carbohydrates (table 3). Mutated cases had a lower intake of polyunsaturated fats (PUFAs) and omega 3 fatty acids (ORs for lowest tertile of intake = 0.19; p for trend <0.03). No significant associations were observed with saturated fats, cholesterol, monounsaturated fats (MUFAs) and omega 6 fatty acids.

Regarding antioxidant dietary components, mutated patients had a lower intake of vitamin E: the adjusted ORs for medium and high tertiles were 0.42 and 0.24, respectively (p for trend = 0.036). Although statistically non-significant, intakes of total carotene and vitamin C were also lower among mutated cases. There was no statistically significant association between the reported intake of vitamin D and K-*ras* status. Concerning the dietary methyl donors examined (vitamin B6, vitamin B12 and folate), intake of vitamin B6 was also lower among mutated cases. No association was observed with folate. The energy-, age- and sex-adjusted ORs for the medium and high categories of calcium intake were 2.85 and 3.79, respectively (p for trend = 0.030) (table 3). When milk and other dairy products were included in the model the calcium ORs became attenuated and statistically non-significant. This was also the case when the ORs for calcium were adjusted for coffee. Inclusion in the model of other food groups and nutrients did not materially modify these estimates.

DISCUSSION

Patients with a K-*ras* mutated tumour reported a higher intake of dairy products, and lower intakes of vitamin E, PUFAs and omega 3 fatty acids than patients with K-*ras* wild-type tumours. Dietary factors were hence differentially related with K-*ras* mutated and K-*ras* wild-type EPC. These findings support the notion of aetiological heterogeneity of pancreatic cancer.[10 11]

The occurrence of pancreatic cancer may be influenced by environmental factors.[1–3] Dietary factors are among possible causal agents, although findings are inconsistent, and dietary components and biological mechanisms remain unclear.[5–9] Disagreement among studies might partly be due to the fact that they analysed all cases of pancreatic cancer combined; it is likely, however, that wild-type K-*ras* EPC arise through pathways distinct from tumours that harbour K-*ras* mutations.[1–4 10–12]

The association between K-*ras* mutations and dairy products might be coherent with previously unconnected and still tentative findings on diet, organochlorine compounds and pancreatic cancer.[1 3 37] A positive association between butter and saturated-fat intake and pancreatic cancer risk was found in a prospective study in Finland;[38] a case-control study in Louisiana also found a positive relation with consumption of dairy foods.[39] However, inverse and null effects of dairy products were seen in other traditional studies, and the risk associated with fat intake was inconsistent.[5 40 41] Fat foods are known to contain highly lipophilic environmental chemical agents, including organochlorine compounds; some 80–90% of human exposure to organochlorines occurs through food, including butter and other dairy products.[42–52] Some organochlorines have been associated with risk of pancreatic cancer[1 53] and with K-*ras* mutations in exocrine pancreatic cancer.[10] Given the age of most patients in our study, it is likely that the intake of dairy products quantified by our questionnaire was correlated with intake at the time when organochlorines were commonly present in these foods. Although use of organochlorine pesticides was restricted in Spain in the early 1980s, recent studies continue to find organochlorine residues in dairy products and other food groups.[10 45–52]

We previously reported that pancreatic cancer patients with mutations in K-*ras* drank more coffee than cases without a mutation, with a dose-response relation;[15] these analyses did not include dietary information. Jacobsen and Heuch showed independently from us that our results were ''consistent with an overall lack of association between coffee drinking and the risk of pancreatic cancer''.[18] The conclusion of an overall lack of association and the hypothesis of an association with K-*ras* mutations are also supported by other lines of evidence.[1 3 15–18 54–58] Evidence for an association between coffee consumption and K-*ras* mutations in exocrine pancreatic cancer is also present in the study by Slebos *et al*:[17 54] cases with a K-*ras* mutated tumour were almost three times more likely to be in the upper category of total coffee consumption than cases without a mutation (OR 2.78, p = 0.11); in fact, the study[17] probably suffered from biases that tended to dilute the association.[1 54]

Might coffee drinking and dairy products mutually confound their respective associations with K-*ras*? Not according to the results presented here: consumption of coffee and dairy products were independently associated with an increased odds of having a K-*ras* mutated pancreatic cancer. Furthermore, after adjusting for calcium the ORs for milk and other dairy products increased significantly, while the ORs for coffee did not change. This suggests that the association between dairy products and K-*ras* mutations is not confounded by calcium. It also suggests the need to consider the hypothesis that environmental contaminants might be involved.[1 10] Furthermore, the association between calcium intake and K-*ras* mutations was attenuated when dairy products were included in the model. Non-genotoxic or perhaps epigenetic

Table 1 Consumption of food groups among cases of exocrine pancreatic cancer with and without mutations in the K-*ras* oncogene

		K-*ras*			Adjusted OR†		Adjusted OR‡	
	All cases (n = 107)	Mutated (n = 83)	Wild-type (n = 24)	p Value§	OR (95% CI)	p Value ¶	OR (95% CI)	p Value ¶
Milk and other dairy products*								
Non-daily	18 (16.8)	10 (12.0)	8 (33.3)	0.026	1.00	0.008	1.00	0.005
Daily	89 (83.2)	73 (88.0)	16 (66.7)		6.30 (1.56–25.41)		7.58 (1.73–33.25)	
Butter								
Low‡‡	68 (63.6)	49 (59.0)	19 (79.2)	0.063**	1.00	0.049	1.00	0.048
Medium	11 (10.3)	9 (10.8)	2 (8.3)		1.77 (0.34–9.24)		1.74 (0.32–9.40)	
High	28 (26.2)	25 (30.1)	3 (12.5)		3.45 (0.89–13.36)		3.59 (0.90–14.51)	
All dairy products								
Low§§	17 (15.9)	9 (10.8)	8 (33.3)	0.008**	1.00	0.003	1.00	0.002
Medium	63 (58.9)	50 (60.2)	13 (54.2)		6.15 (1.50–25.24)		7.43 (1.67–33.05)	
High	27 (25.2)	24 (28.9)	3 (12.5)		15.53 (2.35–102.40)		19.68 (2.64–146.51)	
Coffee								
Non-regular drinker	18 (16.8)	10 (12.0)	8 (33.3)	0.026	1.00	0.011	1.00	0.006
Regular drinker	89 (83.2)	73 (88.0)	16 (66.7)		5.27 (1.44–19.30)		6.99 (1.71–28.66)	
0 cups per week	18 (17.1)	10 (12.3)	8 (33.3)	0.038**	1.00	0.014	1.00	0.004
1–7 cups per week	28 (26.7)	22 (27.2)	6 (25.0)		4.07 (0.96–17.27)		4.30 (0.92–20.08)	
8–14 cups per week	27 (25.7)	22 (27.2)	5 (20.8)		5.75 (1.13–29.12)		8.75 (1.48–51.57)	
⩾15 cups per week	32 (30.5)	27 (33.3)	5 (20.8)		6.33 (1.39–28.88)		11.15 (2.03–61.23)	
Raw vegetables								
Low§§	17 (15.9)	12 (14.5)	5 (20.8)	0.171	1.00	0.605	1.00	0.668
Medium	35 (32.7)	31 (37.3)	4 (16.7)	0.645**	3.58 (0.77–16.55)	0.103††	3.24 (0.60–15.53)	0.211††
High	55 (51.4)	40 (48.2)	15 (62.5)		1.08 (0.30–3.89)		1.09 (0.26–4.53)	
Cooked vegetables								
Low§§	31 (29.0)	28 (33.7)	3 (12.5)	0.112	1.00	0.096	1.00	0.136
Medium	49 (45.8)	35 (42.2)	14 (58.3)	0.124**	0.24 (0.06–0.95)	0.074††	0.20 (0.05–0.92)	0.077††
High	27 (25.2)	20 (24.1)	7 (29.2)		0.26 (0.06–1.21)		0.25 (0.05–1.33)	
All vegetables								
Low§§	46 (43.0)	38 (45.8)	8 (33.3)	0.493	1.00	0.228	1.00	0.369
Medium	40 (37.4)	30 (36.1)	10 (41.7)	0.271**	0.55 (0.17–1.74)		0.60 (0.17–2.15)	
High	21 (19.6)	15 (18.1)	6 (25.0)		0.47 (0.13–1.68)		0.54 (0.14–2.13)	
Fruits								
Low§§	8 (7.5)	7 (8.4)	1 (4.2)	0.765	1.00	0.583	1.00	0.536
Medium	15 (14.0)	11 (13.3)	4 (16.7)	0.712**	0.39 (0.04–4.41)	0.706††	0.38 (0.03–5.08)	0.705††
High	84 (78.5)	65 (78.3)	19 (79.2)		0.45 (0.04–4.32)		0.40 (0.04–4.52)	
Vegetables & fruits								
Low§§	33 (30.8)	28 (33.7)	5 (20.8)	0.397	1.00	0.173	1.00	0.219
Medium	32 (29.9)	25 (30.1)	7 (29.2)	0.232**	0.51 (0.13–1.98)	0.084††	0.44 (0.10–1.97)	0.083††
High	42 (39.3)	30 (36.1)	12 (50.0)		0.31 (0.08–1.22)		0.28 (0.06–1.26)	
Vegetable oil								
Low§§	7 (6.5)	6 (7.2)	1 (4.2)	0.888	1.00	0.320	1.00	0.182
Medium	8 (7.5)	7 (8.4)	1 (4.2)	0.411**	1.20 (0.06–24.53)		0.57 (0.02–15.05)	
High	92 (86.0)	70 (84.3)	22 (91.7)		0.46 (0.05–4.38)		0.24 (0.02–2.95)	
Lard								
Low‡‡	91 (85.0)	72 (86.7)	19 (79.2)	0.549	1.00	0.376	1.00	0.575
Medium	10 (9.3)	7 (8.4)	3 (12.5)	0.364**	0.63 (0.15–2.69)		0.61 (0.12–3.12)	0.807††
High	6 (5.6)	4 (4.8)	2 (8.3)		0.52 (0.08–3.13)		0.73 (0.09–5.73)	
Meat, chicken & organs								
Low§§	8 (7.5)	7 (8.4)	1 (4.2)	0.715	1.00	0.282	1.00	0.376
Medium	61 (57.0)	48 (57.8)	13 (54.2)	0.377**	0.52 (0.06–4.74)		0.83 (0.08–8.07)	
High	38 (35.5)	28 (33.7)	10 (41.7)		0.33 (0.03–3.40)		0.48 (0.04–5.32)	
Sausages & cured meats								
Low§§	35 (32.7)	30 (36.1)	5 (20.8)	0.369	1.00	0.109	1.00	0.147
Medium	36 (33.6)	27 (32.5)	9 (37.5)	0.176**	0.48 (0.14–1.65)		0.40 (0.11–1.53)	
High	36 (33.6)	26 (31.3)	10 (41.7)		0.34 (0.09–1.31)		0.35 (0.08–1.54)	
Meat & sausages								
Low§§	27 (25.2)	23 (27.7)	4 (16.7)	0.207	1.00	0.027	1.00	0.021
Medium	39 (36.4)	32 (38.6)	7 (29.2)	0.086**	0.68 (0.17–2.73)		0.53 (0.11–2.45)	
High	41 (38.3)	28 (33.7)	13 (54.2)		0.21 (0.05–0.93)		0.15 (0.03–0.86)	
Fish & shellfish								
Low§§	27 (25.2)	21 (25.3)	6 (25.0)	0.466	1.00	0.465	1.00	0.380
Medium	69 (64.5)	55 (66.3)	14 (58.3)	0.525**	1.08 (0.36–3.25)	0.467††	1.05 (0.31–3.53)	0.179††
High	11 (10.3)	7 (8.4)	4 (16.7)		0.43 (0.08–2.21)		0.36 (0.06–2.09)	
Eggs								
Low¶¶	41 (38.3)	33 (39.8)	8 (33.3)	0.639	1.00	0.537	1.00	0.702
High	66 (61.7)	50 (60.2)	16 (66.7)		0.74 (0.28–1.95)		0.81 (0.28–2.34)	
Bread								
Non-daily	5 (4.7)	3 (3.6)	2 (8.4)	0.312	1.00	0.356	1.00	0.363
Daily	102 (95.3)	80 (96.4)	22 (91.7)		2.63 (0.36–19.36)		2.94 (0.30–29.27)	
Potatoes								
Low§§	10 (9.3)	6 (7.2)	4 (16.7)	0.263	1.00	0.722	1.00	0.311
Medium	47 (43.9)	39 (47.0)	8 (33.3)	0.730**	3.44 (0.73–16.10)	0.271††	3.76 (0.66–21.37)	0.321††
High	50 (46.7)	38 (45.8)	12 (50.0)		2.17 (0.40–11.62)		3.80 (0.56–25.90)	

Table 1 Continued

		K-*ras*			Adjusted OR†		Adjusted OR‡	
	All cases (n = 107)	Mutated (n = 83)	Wild-type (n = 24)	p Value§	OR (95% CI)	p Value ¶	OR (95% CI)	p Value ¶
Cereals & legumes								
Low‡‡	9 (8.4)	5 (6.0)	4 (16.7)	0.253	1.00	0.423	1.00	0.337
Medium	48 (44.9)	39 (47.0)	9 (37.5)	0.426**	3.55 (0.78–16.25)	0.276††	4.45 (0.77–25.73)	0.245††
High	50 (46.7)	39 (47.0)	11 (45.8)		2.94 (0.64–13.45)		3.65 (0.68–19.66)	

Values in parentheses are column percentages.
The first category of each variable is the reference category (OR 1.00).
*Excluding butter.
†Odds ratio adjusted for total energy, age and sex.
‡Odds ratio adjusted for total energy, age, sex, smoking, alcohol and coffee (dairy products not adjusted for coffee, see table 2).
§Unless otherwise specified, p value derived from Fisher's exact test.
¶Unless otherwise specified, p value derived from the multivariate analogue of Mantel's extension test for linear trend.
**Mantel–Haenszel's χ^2 test for linear trend.
††Likelihood ratio test.
‡‡Low: rarely or never; medium: several times per month; high: several times per week or daily.
§§Low: rarely or never, or several times per month; medium: several times per week; high: daily.
¶¶Low: rarely or never, or several times per month; high: several times per week, or daily.

mechanisms might explain the observed associations.[1 3 10 59] For instance, a well-known role of caffeine in carcinogenesis is interference with DNA repair and modulation of apoptosis.[15 58] These two mechanisms have also been suggested as potential non-genotoxic pathways through which organochlorine compounds could modulate K-*ras* mutations or effects.[1 3 10]

Although meat and sausages are also potential fatty food sources of organochlorine compounds, consumption of these foods was higher among patients with wild-type K-*ras* tumours. A similar result has been reported in colorectal adenomas[26] and colorectal cancer.[24] Experiments in animal models reported that heterocyclic amines, formed during meat cooking, may select for K-*ras* mutation-negative lesions.[60] Also, in rat colon and mammary tumours induced by heterocyclic amines *ras* alterations are rarely detected.[61 62] Our findings are hence coherent with the view that in pancreatic carcinogenesis heterocyclic amines or other carcinogenic compounds present in meats tend to select for K-*ras* negative tumours.

Table 2 Consumption of dairy products and coffee among cases of exocrine pancreatic cancer with and without K-*ras* mutations. Mutually-adjusted odds ratios.

	OR† (95% CI)	p value‡
Milk and other dairy products*		
Non-daily	1.00	0.040
Daily	5.10 (1.06–24.52)	
Butter		
Low¶	1.00	0.096
Medium	3.39 (0.54–21.42)	0.185§
High	2.61 (0.61–11.24)	
All dairy products		
Low**	1.00	0.023
Medium	5.38 (1.12–25.85)	
High	11.56 (1.36–98.55)	
Coffee		
Non-regular drinker	1.00	0.043
Regular drinker	4.66 (1.05–20.71)	
0 cups per week	1.00	0.034
1–7 cups per week	3.30 (0.65–16.79)	
8–14 cups per week	5.53 (0.89–34.39)	
⩾15 cups per week	6.56 (1.10–39.10)	

The first category of each variable is the reference category (OR 1.00).
*Excluding butter.
†Odds ratio adjusted for total energy, age, sex, alcohol and smoking. Dairy products and butter further adjusted for coffee. Coffee further adjusted for milk and other dairy products.
‡Unless otherwise specified, p value derived from the multivariate analogue of Mantel's extension test for linear trend.
§Likelihood ratio test.
¶Low: rarely or never; medium: several times per month; high: several times per week or daily.
**Low: rarely or never, or several times per month; medium: several times per week; high: daily.

Although in animal models caloric restriction may decrease the activity of some oncogenes and activate silenced tumour suppressor genes,[63 64] we did not find differences in energy intake between K-*ras* mutated and wild-type cases. This suggests that in pancreatic cancer K-*ras* activation or clonal selection of K-*ras* mutated cells might be independent of caloric intake.[2 13] While the percentage of calories covered by the bFFQ is uncertain,[28] absolute values for total calories were plausible for the type of participants, given their age and sex. Our main purpose was to obtain estimates to classify participants properly according to their answers to the bFFQ; if a misclassification was present, it was more likely to be non-differential.

Some epidemiological studies observed a protective effect of vitamin C on the development of pancreatic cancer, but the associations with intake of other dietary antioxidants such as carotenoids and vitamin E were weak and inconsistent.[1 5] We found a higher intake of vitamin E in K-*ras* wild-type than in mutated cases. This finding agrees with previous experimental studies in animal models of carcinogenesis, which reported a protective effect of vitamin E against K-*ras* mutations.[27] The proposed mechanisms for the anti-carcinogenic effect of vitamin E include the inhibition of lipid peroxidation and formation of its reactive products. It has been shown that the pancreas suffers DNA damage from oxidative stress and lipid peroxidation.[1 3] Vitamin E may reduce the binding of reactive products to DNA, therefore preventing oxidative damage to DNA. Also, vitamin E inhibits the formation of powerful mutagenic nitric oxide species such as nitrosamines, which have been related with codon 12 K-*ras* mutations in pancreatic carcinomas.[65] Although vitamin D and its analogues exhibit potent anti-tumour effects in several tissues, including the pancreas,[8 9] our results suggest that vitamin D intake is not involved in pancreatic carcinogenesis through the K-*ras* mutation pathway.

Our results might also suggest that some types of fat intervene in regulating K-*ras* expression or growth of K-*ras* mutated cells. In most animal studies, omega 6 fatty acids have a stronger enhancing effect on pancreatic carcinogenesis than saturated fat, while omega 3 fatty acids (for example, certain fish oils) are inhibitory to tumour growth.[66 67] In vitro studies reported that specific dietary fats differentially regulate gene

Table 3 Energy-adjusted nutrient consumption in exocrine pancreatic cancer cases with and without K-*ras* mutations. Tertiles of energy-adjusted intakes of nutrients

		K-*ras*			Adjusted OR*		Adjusted OR†	
	All cases (n = 107)	Mutated (n = 83)	Wild-type (n = 24)	p Value‡	OR (95% CI)	p Value §	OR (95% CI)	p Value §
Energy intake (Kcal)								
Mean (SD)	1761.1 (328.7)	1763.8 (310.4)	1751.8 (392.8)	0.891¶				
Median	1745.1	1745.1	1728.7					
<1536.1	27 (25.2)	17 (20.5)	10 (41.7)	0.223**	1.00	0.222	1.00	0.982
1536.1–1828.5	34 (31.8)	30 (36.1)	4 (16.6)	0.069	4.60 (1.22–17.35)	0.060††	3.38 (0.78–14.66)	0.155††
>1828.5	46 (43.0)	36 (43.4)	10 (41.7)		2.10 (0.73–6.02)		1.16 (0.31–4.41)	
Total protein (g)								
<70.58	32 (29.9)	26 (31.3)	6 (25.0)	0.504**	1.00	0.493	1.00	0.313
70.58–79.0	44 (41.1)	34 (41.0)	10 (41.7)	0.767	0.77 (0.25–2.43)		0.99 (0.29–3.44)	
>79.0	31 (29.0)	23 (27.7)	8 (33.3)		0.65 (0.19–2.21)		0.51 (0.13–1.92)	
Total fat (g)								
<69.41	30 (28.0)	26 (31.3)	4 (16.7)	0.429**	1.00	0.427	1.00	0.506
69.41–75.41	37 (34.6)	26 (31.3)	11 (45.8)	0.290	0.33 (0.09–1.23)	0.228††	0.24 (0.06–0.99)	0.103††
>75.41	40 (37.4)	31 (37.4)	9 (37.5)		0.53 (0.15–1.92)		0.55 (0.12–2.44)	
Saturated fat (g)								
<20.96	30 (28.0)	23 (27.7)	7 (29.2)	0.296**	1.00	0.296	1.00	0.588
20.96–23.54	40 (37.4)	28 (33.7)	12 (50.0)	0.221	0.64 (0.20–2.04)	0.185††	0.47 (0.13–1.72)	0.198††
>23.54	37 (34.6)	32 (38.6)	5 (20.8)		1.89 (0.53–6.77)		1.43 (0.34–6.08)	
MUFA (g)								
<30.65	32 (29.9)	26 (31.3)	6 (25.0)	0.504**	1.00	0.493	1.00	0.315
30.65–34.25	44 (41.1)	34 (41.0)	10 (41.7)	0.767	0.77 (0.25–2.43)		0.99 (0.29–3.44)	
>34.25	31 (29.0)	23 (27.7)	8 (33.3)		0.65 (0.19–2.21)		0.51 (0.13–1.92)	
PUFA (g)								
<11.55	36 (33.6)	32 (38.6)	4 (16.6)	0.038**	1.00	0.024	1.00	0.027
11.55–12.36	40 (37.4)	30 (36.1)	10 (41.7)	0.090	0.40 (0.11–1.48)		0.34 (0.08–1.48)	
>12.36	31 (29.0)	21 (25.3)	10 (41.7)		0.22 (0.05–0.93)		0.19 (0.04–0.94)	
Omega 3 fats (g)								
<1.08	38 (35.5)	32 (38.5)	6 (25.0)	0.044**	1.00	0.035	1.00	0.024
1.08–1.20	43 (40.2)	35 (42.2)	8 (33.3)	0.093	0.81 (0.25–2.64)		0.75 (0.22–2.64)	
>1.20	26 (24.3)	16 (19.3)	10 (41.7)		0.26 (0.08–0.91)		0.19 (0.05–0.81)	
Omega 6 fats (g)								
<10.23	35 (32.7)	28 (33.7)	7 (29.2)	0.222**	1.00	0.193	1.00	0.098
10.23–11.03	42 (39.3)	35 (42.2)	7 (29.2)	0.242	1.44 (0.43–4.76)	0.115††	1.65 (0.43–6.35)	0.059††
>11.03	30 (28.0)	20 (24.1)	10 (41.6)		0.31 (0.08–1.27)		0.30 (0.06–1.45)	
Cholesterol (mg)								
<249.8	25 (23.4)	21 (25.4)	4 (16.6)	0.471**	1.00	0.456	1.00	0.372
249.8–312.0	41 (38.3)	31 (37.3)	10 (41.7)	0.669	0.57 (0.15–2.11)		0.75 (0.19–3.05)	
>312.0	41 (38.3)	31 (37.3)	10 (41.7)		0.58 (0.16–2.11)		0.55 (0.14–2.19)	
Carbohydrates (g)								
<162.25	38 (35.5)	29 (34.9)	9 (37.5)	0.415**	1.00	0.394	1.00	0.443
162.25–184.30	41 (38.3)	30 (36.2)	11 (45.8)	0.494	0.82 (0.29–2.30)	0.390††	0.82 (0.26–2.57)	0.448††
>184.30	28 (26.2)	24 (28.9)	4 (16.7)		1.96 (0.53–7.28)		1.96 (0.45–8.60)	
Fibre (g)								
<14.85	47 (43.9)	38 (45.8)	9 (37.5)	0.941**	1.00	0.946	1.00	0.814
14.85–17.56	32 (29.9)	22 (26.5)	10 (41.7)	0.386	0.51 (0.18–1.46)	0.351††	0.40 (0.12–1.34)	0.079††
>17.56	28 (26.2)	23 (27.7)	5 (20.8)		1.11 (0.33–3.77)		1.36 (0.34–5.41)	
Carotene (RE)								
<372.0	40 (37.4)	34 (41.0)	6 (25.0)	0.248**	1.00	0.209	1.00	0.192
372.0–650.0	40 (37.4)	29 (34.9)	11 (45.8)	0.391	0.43 (0.13–1.36)	0.293††	0.28 (0.07–1.03)	0.131††
>650.0	27 (25.2)	20 (24.1)	7 (29.2)		0.45 (0.13–1.62)		0.40 (0.10–1.63)	
Retinol (RE)								
<436.3	39 (36.4)	31 (37.4)	8 (33.3)	0.820**	1.00	0.821	1.00	0.588
436.3–581.0	37 (34.6)	28 (33.7)	9 (37.5)	0.957	0.79 (0.27–2.36)	0.917††	0.94 (0.28–3.07)	0.845††
>581.0	31 (29.0)	24 (28.9)	7 (29.2)		0.89 (0.28–2.84)		0.70 (0.20–2.46)	
Vitamin E (mg)								
<9.29	50 (46.7)	42 (50.6)	8 (33.3)	0.076**	1.00	0.063	1.00	0.036
9.29–10.43	34 (31.8)	26 (31.3)	8 (33.3)	0.201	0.57 (0.18–1.79)		0.42 (0.12–1.45)	
>10.43	23 (21.5)	15 (18.1)	8 (33.3)		0.31 (0.09–1.08)		0.24 (0.06–0.98)	
Vitamin C (mg)								
<125.5	50 (46.8)	42 (50.6)	8 (33.3)	0.375**	1.00	0.356	1.00	0.276
125.5–157.0	30 (28.0)	20 (24.1)	10 (41.7)	0.177	0.34 (0.11–1.05)	0.168††	0.14 (0.04–0.57)	0.014††
>157.0	27 (25.2)	21 (25.3)	6 (25.0)		0.64 (0.18–2.24)		0.57 (0.14–2.38)	
Vitamin D (μg)								
<4.23	41 (38.3)	30 (36.1)	11 (45.8)	0.402**	1.00	0.351	1.00	0.882
4.23–5.13	39 (36.4)	31 (37.3)	8 (33.3)	0.731	1.42 (0.48–4.15)	0.348††	0.94 (0.29–3.03)	0.882††
>5.13	27 (25.2)	22 (26.5)	5 (20.8)		1.78 (0.48–6.56)		1.16 (0.27–4.95)	
Vitamin B6 (mg)								
<1.52	35 (32.7)	31 (37.3)	4 (16.7)	0.047**	1.00	0.035	1.00	0.098
1.52–1.71	44 (41.1)	33 (39.8)	11 (45.8)	0.108	0.35 (0.10–1.27)		0.32 (0.07–1.34)	
>1.71	28 (26.2)	19 (22.9)	9 (37.5)		0.24 (0.06–0.94)		0.27 (0.06–1.24)	
Vitamin B12 (μg)								
<6.95	38 (35.5)	32 (38.6)	6 (25.0)	0.266**	1.00	0.258	1.00	0.199
6.95–9.23	43 (40.2)	32 (38.6)	11(45.8)	0.468	0.54 (0.18–1.65)		0.63 (0.19–2.07)	
>9.23	26 (24.3)	19 (22.9)	7 (29.2)		0.50 (0.14–1.75)		0.42 (0.11–1.61)	

Table 3 Continued

	All cases (n = 107)	K-*ras* Mutated (n = 83)	K-*ras* Wild-type (n = 24)	p Value‡	Adjusted OR* OR (95% CI)	p Value §	Adjusted OR† OR (95% CI)	p Value §
Folate (μg)								
<235.0	43 (40.2)	33 (39.8)	10 (41.6)	0.917**	1.00	0.905	1.00	0.862
235.0–282.35	36 (33.6)	29 (34.9)	7 (29.2)	0.876	1.25 (0.41–3.79)	0.865††	0.77 (0.22–2.73)	0.923††
>282.35	28 (26.2)	21 (25.3)	7 (29.2)		0.90 (0.30–2.76)		0.91 (0.26–3.15)	
Calcium (mg)								
<778.0	43 (40.2)	29 (34.9)	14 (58.3)	0.070**	1.00	0.030	1.00	0.166
7778.0–927.9	38 (35.5)	32 (38.6)	6 (25.0)	0.145	2.85 (0.93–8.77)		1.69 (0.49–5.82)	
>927.9	26 (24.3)	22 (26.5)	4 (16.7)		3.79 (0.91–15.87)		2.87 (0.60–13.75)	

Values in parentheses are column percentages.
RE, retinol equivalents (1 RE = 1 μg = 5 IU of vitamin A).
*Odds ratio adjusted for total energy, age and sex.
†Odds ratio adjusted for total energy, age, sex, smoking, alcohol and coffee.
‡Unless otherwise specified, p value derived from Fisher's exact test.
§Unless otherwise specified, p value derived from multivariate analogue of Mantel's extension test for linear trend.
¶Student's t test.
**Mantel-Haenszel's χ^2 test for linear trend.
††Likelihood ratio test.

expression,[68 69] which is in accordance with findings in the present study. Dietary fats are thought to modulate *ras* functions during the promotion and progression of colon cancer, resulting in decreased expression of *ras*.[68 70] Other molecular mechanisms may contribute to the benefits of omega-3 fatty acids, including limitation of tumour cell proliferation, increase of apoptosis, promotion of cell differentiation, modulation of *ras* proteins and protein kinase C activity, and possibly limitations of angiogenesis.[3 71 72]

Clearly, some of our estimates were statistically imprecise. This was largely because of the small number of K-*ras* wild-type cases, a consistent finding in EPC.[1–3] Also, a relatively high number of dietary factors were compared. Hence, both false negatives and false positives may have occurred. Nonetheless, the direction of the association with K-*ras* status was largely consistent for food groups and nutrients that are commonly correlated (for example, PUFA and vegetables and fruits, calcium and dairy products). Although undoubtedly small, to date this is the largest study with environmental and genetic information in pancreatic cancer, and only one of two such studies based on personal interviews with patients[16 17]; the other study included 61 cases (15 K-*ras* wild-type cases and 46 K-*ras* mutated cases).[17] Ours is the first study to analyse diet and K-*ras* activation in human pancreatic cancer, and one of fewer than 10 on *ras* mutations and diet in all cancers. We achieved a high response rate, and over 80% of patients were interviewed during hospital stay, near the time of diagnosis. The proportion of cases with both genetic and dietary data is also high for EPC.[54] Lack of differences between patients included and excluded further argues against selection bias.[10 15] In pancreatic and other cancers selection biases are proving to be particularly difficult to overcome by studies using biomarkers of environmental exposures and genetic information.[54 73]

Our study did not directly estimate the effects of food and nutrient intakes on the risk of pancreatic cancer, as many other studies have done;[5–9] no such studies assessed gene/environment interactions. Indeed, outside colorectal neoplasms[19–26] it is still fairly exceptional to find case-case-control studies assessing gene/environment interactions and relatively free from selection and information bias (that is, including a large proportion of the original incident cases in analyses that involve genes, biomarkers of environmental exposures, clinical information, and data from patient interviews). Retrieving tumour DNA from hospitals seems a major obstacle.[1]

In addition to chance, several methodological features may have influenced results. The brief questionnaire clearly presented a limited ability to disaggregate higher intakes of some food groups, such as milk and other dairy products, fresh vegetables, bread and fruit. We did not pretend that the correlation with simulated intake would be a validation study for the bFFQ.[28] Rather, with that exercise we tried to assess whether the bFFQ was an acceptable instrument to classify participants according to food and nutrient intakes, including energy intake. By assuming that specific food intakes from a nutritional survey carried out with a similar population of the same region could be applied to our study population, we observed plausible values for food and nutrient intakes that we considered good enough to classify participants with an acceptable degree of certainty.[28] Despite its limitations the questionnaire seemed useful to identify subjects with very low intakes of the above-mentioned food groups and related nutrients. Some nutrient intakes may have been underestimated, and a certain degree of misclassification is inevitable when assessing diet in patients who are severely ill.[28 30] Furthermore, misclassification was most likely non-differential with respect to K-*ras* status; hence, better dietary measurements seem more likely to reveal stronger than weaker associations.

In conclusion, exposure to specific dietary components or contaminants could contribute to the occurrence or persistence of K-*ras* mutations in human exocrine pancreatic cancer. Findings might have implications as well for other neoplasms with a high frequency of mutations in the K-*ras* oncogene (for example, colon and lung adenocarcinomas). Nonetheless, because this is only the first study on diet and K-*ras* mutations in pancreatic cancer, studies free from selection and information bias, and with the power to refute or to confirm the results are required before mechanistic, clinical and public health implications—if any—are considered.

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What is already known

- Over 20 years of research have shown that *ras* genes can be involved in the initiation of carcinogenesis and are critical DNA targets for chemical carcinogens, and that somatic (acquired) mutations in the K-*ras* oncogene are an early and fundamental event in the aetiopathogenesis of exocrine pancreatic cancer.
- K-*ras* mutations are also common in other human cancers, as lung and colon adenocarcinomas; they are the most frequent abnormality of oncogenes in human cancer.
- Research on lifestyle and environmental influences upon the occurrence and persistence of *ras* mutations in humans is scant.
- Fat foods contain lipophilic environmental chemical agents, like organochlorine compounds; most human exposure to organochlorines occurs through food, including dairy products.

What this paper adds

- This is the first study on the relation between dietary factors and K-*ras* mutations in human exocrine pancreatic cancer. It is the largest study with molecular environmental and genetic information in this neoplasm.
- Results suggest that exposure to specific dietary components may influence the occurrence or persistence of K-*ras* mutations in pancreatic cancer.
- Findings may have mechanistic implications for other neoplasms with a high frequency of K-*ras* mutations.
- Findings are relevant for scientists working on human carcinogenesis, diet or pancreatic cancer; they contribute to the scant literature on the role of gene/environment interactions in the poorly understood aetiopathogenesis of this lethal disease.

Policy implication

- Studies free from selection and information biases, and with the power to refute or to confirm the results are required before policy implications are considered.

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