SHORT COMMUNICATION

Animal products and K-ras codon 12 and 13 mutations in colon carcinomas


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K-ras gene mutations (codons 12 and 13) were determined by PCR-based mutant allele-specific amplification (MASA) in tumour tissue of 185 colon cancer patients: 36% harboured mutations, of which 82% were located in codon 12. High intakes of animal protein, calcium and poultry were differently associated with codon 12 and 13 mutations: odds ratios (OR) and 95% confidence intervals (95% CI) for codon 12 versus codon 13 were 9.0 (2.0–42), 4.1 (1.4–12) and 15 (1.4–160), respectively. In case–control comparisons, high intakes of animal protein and calcium were positively associated with colon tumours harbouring codon 12 mutations [for animal protein per 17 g, OR (95% CI) = 1.5 (1.0–2.1); for calcium per 459 mg, 1.2 (0.9–1.6)], while inverse associations were observed for tumours with K-ras mutations in codon 13 [for animal protein 0.4 (0.2–1.0); for calcium 0.6 (0.3–1.2)]. Transition and transversion mutations were not differently associated with these dietary factors. These data suggest a different aetiology of colon tumours harbouring K-ras codon 12 and 13 mutations.

Diets rich in meat, saturated fat and animal protein may increase colon cancer risk (1). During colon carcinogenesis somatic mutations accumulate (2), but the role of diet in their aetiology is still a matter of debate. About 50% of colon tumours harbour single point mutations in the Kirsten ras oncogene (3). Diet-related carcinogens, such as heterocyclic amines from heavily cooked meat, may induce K-ras mutations (4). Dietary factors may also affect clonal selection by modifying growth of tumours harbouring K-ras mutations (5,6). About 80% of K-ras mutations in colon tumours are located in codon 12, while mutations in codon 13 are observed in ±10% (3,7). Mutations in codon 12 are either more likely to occur or are more likely to exert a selective growth advantage when they occur (3). Most mutations in codon 13 are transition mutations, while in codon 12 G→A transition as well as G→T transversion mutations are most common (8). We explored whether the consumption of products of animal origin and their constituents are associated with the occurrence of K-ras mutations in colon tumours.

A population-based case–control study on diet and colon cancer was conducted in The Netherlands. Methods have been published in detail (9). Cases (n = 204) were men and women with colon cancer, newly diagnosed at surgery. Controls (n = 259), frequency matched to the cases by age (5 year intervals), gender, region and degree of urbanization, were recruited randomly by general practitioners of the cases. All subjects were Caucasian, up to 75 years old at time of diagnosis and did not have previous cancer, polyposis coli or inflammatory bowel disease. Except for a more favourable Dukes’ stage in cases, participants did not differ importantly from non-participants. From 185 cases, paraffin-embedded colon tumour tissue was available, collected close to the interview and before chemo- or radiotherapy started. From 19 patients, tissue was unavailable for administrative reasons. Usual dietary habits were assessed by an interview-based questionnaire. The consumption frequency per month, number of months during which the item was used, number of portions per consumption and portion size of 289 food items were inquired into. Average daily intake of nutrients was calculated using the Dutch National Food Table (10). Nutrient intake was adjusted for energy by the residual method (11). Previous analyses of this case–control study showed an increased risk of colon cancer with high meat and calcium intake (9,12).

DNA was isolated as described elsewhere (13). Codons 12 and 13 of K-ras were examined by mutant allele-specific amplification (MASA) as described by Hasegawa et al. (14). For all PCR experiments 5′-CCAGGTCTCGTAA-GAAAC-3′ was used as reverse primer. MASA–PCR was performed as follows. PCR reaction mixture (40 µl) consisted of 300 ng purified DNA, 20 mmol/l (NH4)2SO4, 75 mmol/l Tris–HCl (pH 9.0), 0.01% Tween, 200 µmol/l dNTPs, 0.4 µmol/l reverse primer, 2.5 mmol/l MgCl2. After an initial denaturation step of 3 min at 94°C, mixture 2 (10 µl), which consisted of 0.3 U of Thermoperfect Plus DNA polymerase (Integro BV, Zaandam, The Netherlands) and 20 pmol of each of the forward primers, was added immediately to mixture 1. PCR was performed for 35 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C and 72°C for 5 min, in a Mastercycler 5330 (Eppendorf Geratebau GmbH, Hamburg, Germany). Following amplification, 15 µl of each reaction mixture was loaded onto a 2% ethidium bromide stained agarose gel and electrophoresed. A second PCR was performed on those DNA samples that were found positive. Single primers that correspond to each of the variant nucleotides of the positive first PCR set were added per incubation. Conditions were the same as in the PCR described above. Since all samples studied contained wild-type K-ras DNA due to the presence of tumour stroma, we used wild-type K-ras DNA as an internal control for DNA quality. In 98% of the samples wild-type K-ras could be easily amplified. To validate the robustness of MASA, sequence analysis was conducted on PCR products of samples with various K-ras mutations. In all cases analysed we found the same mutation by sequencing as was detected by MASA.

The association between diet and K-ras gene mutations was evaluated by comparing the mutation prevalence among cases.
using logistic regression models (15). In addition, odds ratios (OR) and 95% confidence intervals (CI) were calculated separately for mutant cases and wild-type cases versus the population-based control group. Analyses were first conducted in quartiles of dietary exposure. To quantify the associations on a continuous scale, all ORs and 95% CIs were also expressed for the distance between the 75th and 25th percentiles. Results of both approaches pointed to the same nutrients and food groups being related to the K-ras mutations.

All analyses were adjusted for age, sex and total energy intake. Additional adjustment for Dukes' stage, smoking, body mass index and other dietary factors, such as the consumption of vegetables and fruits, did not change the estimates significantly.

Of the 185 colon tumours included in this study, 66 (36%) harboured K-ras mutations: 55 tumours (82%) showed a mutation in codon 12, and 12 (18%) in codon 13. In one tumour, mutations were found in both codons. Of the 55 mutations in codon 12 (wild-type GGT), 23 (42%) were transition mutations, of which GAT (96%) was most common, and 32 (58%) were transversion mutations, of which the GTT mutation (53%) was most frequent. In codon 13 (wild-type GGC), GAC transitions (92%) were most frequent. Cases with and without any K-ras mutations in either codon 12 or codon 13 did not differ significantly with regard to age and gender (data not shown). Proximal tumours (caecum, ascending colon, hepatic flexure and transverse colon) were less frequent among those patients harbouring K-ras mutations in codon 13 (18%) than among those with codon 12 (49%) and K-ras wild-type tumours (46%). Dukes’ stage C and D tumours were relatively more common among those harbouring codon 12 (45%) mutations than among those with codon 13 (38%) and wild-type tumours (32%).

Table I shows the ORs and 95% CIs for case–case comparisons of cases with K-ras mutations versus cases harbouring wild-type tumours (first column), cases with codon 12 mutations versus codon 13 mutations (second column) and cases with transition mutations versus transversion mutations in codon 12 (third column). Overall, K-ras mutational status was not significantly associated with the consumption of animal foods and nutrients. Among mutants, however, substantial differences were observed according to the codon affected. A high intake of protein, especially animal protein, showed a nearly 10-fold risk of K-ras mutations in codon 12 compared with mutations in codon 13. Marked differences were also observed between mutations in codons 12 and 13 for calcium, poultry and dairy products. A comparison of transition mutations with transversion mutations in codon 12 showed no significant animal product-related differences. To further evaluate the observed aetiological heterogeneity between codons 12 and 13, both case groups were compared with the population-based control group. For (animal) protein, calcium and poultry, positive associations were observed with mutations in codon 12: for animal protein (per 17 g), OR (95% CI) 1.5 (1.0–2.1); for calcium (per 459 mg), 1.2 (0.9–1.6); for poultry (per 17 g), 1.2 (0.8–1.6). In contrast, mutations in codon 13 were inversely associated with these dietary factors: animal protein, 0.4 (0.2–1.0); calcium, 0.6 (0.3–1.2); poultry, 0.4 (0.1–1.2).

These data suggest that colon tumours with codon 12 and 13 K-ras mutations are related differently to intake of protein and calcium and to consumption of poultry. No diet-related differences were observed for transition versus transversion mutations.

Although this is the largest study on diet and specific K-ras mutations published to date, it is still of limited size, since collection of both dietary data and tissue blocks is very labour intensive. Furthermore, multiple comparisons may lead to chance findings, especially for the codon and mutation-specific analyses. However, the differences between the two codons appear to be substantial, warranting further investigation. In any retrospective case–control study, selection bias and information bias may affect internal validity. Since cases are unaware of the mutational status of their tumours, systematic errors in dietary recall are less likely to bias study results from our case–case comparisons than from traditional case–control comparisons. However, recall of dietary habits can be influenced by tumour stage or medical treatment influencing appetite. Adjusting case–case comparisons for Dukes’ stage
did not change the estimates significantly, however. The prevalence of K-ras gene mutations (36%) is low as compared with the 35–65% reported by others (3,8,16). This may be due to the lower participation rate among cases with Dukes’ stage C and D tumours, who tend to have higher mutation prevalences. In addition, we evaluated codons 12 and 13, thereby missing codon 61, which may account for 5% of K-ras mutations (7). On the other hand, we assessed K-ras mutations using the MASA method, which is more sensitive than the SSCP method or direct sequencing (17). The spectrum of K-ras mutations is similar to that observed among 2214 colorectal cancer patients: G→A transitions and G→T transversions at the second position of codon 12 were most common, while the GAC mutation occurred most frequently in codon 13 (8). The results obtained from our 185 colon cancer cases do not correspond to a similar study that included 106 colorectal cancer (62 colon) cases (6). Among this Spanish population, an inverse association between high calcium and K-ras mutations was observed (6). In our Dutch population, with a high level and a wide range of calcium intake, we did not observe this inverse association, but did observe an inverse association with codon 13 mutations.

Our data suggest a different dietary aetiology of K-ras codon 12 and 13 mutations. A diet high in meat and animal protein and low in dairy products and calcium might optimize exposure to amines, N-nitro compounds and other carcinogens which could directly lead to bulky DNA adducts and the relevant mutations (1). Possibly, certain carcinogenic compounds preferentially form adducts on codon 12, rather than codon 13. Alternatively, mutations may occur at a similar rate in both codons, but dietary factors, such as protein and calcium, provide an environment more favourable for tumours harbouring mutations in codon 12 than codon 13.

If our findings can be reproduced in larger studies, the diet-related differences between the two main targets for mutations in the K-ras oncogene may provide further insight in the aetiology of colon cancer.

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References


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