Diet, Activity, and Lifestyle Associations with *p53* Mutations in Colon Tumors[1]

Martha L. Slattery,[2] Karen Curtin, K. Ma, Sandra Edwards, Donna Schaffer, Kristen Anderson, and Wade Samowitz

Health Research Center, Department of Family and Preventive Medicine, University of Utah, Salt Lake City, Utah 84108 [M. L. S., K. C., K. M., S. E.; Kaiser Permanente Medical Care Program, Oakland, California 94611-5714 [D. S.]; University of Minnesota, School of Public Health, Minneapolis, Minnesota 55454-1015 [K. A.]; and Department of Surgical Pathology, University of Utah, Salt Lake City, Utah 84132 [W. S.]

Abstract

Inactivation of the *p53* tumor suppressor gene is a common event in the development of colon cancer. We use data collected as part of a multicenter case-control study of colon cancer to evaluate associations between *p53* mutations and diet and lifestyle factors. *p53* mutational status was determined for 1458 incident cases of colon cancer using single-strand conformational polymorphism/sequencing of exons 5–8. We determined associations among those with and without mutations compared with population-based controls ($N = 2410$) and to cases with *p53* mutations compared with cases without *p53* mutations. Associations also were examined by location and function of specific types of *p53* mutations. *p53* mutations were identified in tumors in 47.1% of cases; 81.9% of people with mutations had a missense mutation. Cases with a *p53* mutation were more likely to consume a Western-style diet, compared with controls [odds ratio (OR), 2.03; 95% confidence interval (CI), 1.53–2.69], than were cases who were p53 wild type (Wt), compared with controls (OR, 1.57;95% CI, 1.20–2.06). Specific components of the Western-style diet, including diets with a high glycemic load (mutation *versus* control: OR, 1.48; 95% CI, 1.11–1.98 and Wt *versus* control: OR, 0.98; 95% CI, 0.75–1.28) and diets high in red meat, fast food, and *trans*-fatty acid (mutation *versus* control: OR, 1.92; 95% CI, 1.47–2.50 and Wt *versus* control: OR, 1.39; 95% CI, 1.08–1.80) appeared to be most strongly associated with *p53* mutations. Diets with a high glycemic load (relative to lowest intake) were significantly associated with missense mutations (OR, 1.69; 95% CI, 1.23–2.33 comparing *p53+* to controls and OR, 1.72; 95% CI, 1.19–2.50 comparing cases *p53+* to cases *p53* Wt), as were diets high in red meat, fast food, and *trans*-fatty acids (OR, 1.92; 95% CI, 1.14–2.56 comparing p53+ to controls and OR, 1.40; 95% CI, 1.00–1.98 comparing cases *p53+* to cases *p53* Wt). Physical inactivity, large body mass index, cigarette smoking, using aspirin/nonsteroidal anti-inflammatory drugs, and other dietary factors appeared to be comparably associated with colon cancer in those with and without *p53* mutations. These data suggest that components of a Western-style diet such as high consumption of red meat and foods that increase glycemic load are associated with a *p53* disease pathway.

Introduction

The *p53* tumor suppressor gene is involved in numerous cellular processes, including induction of apoptosis and cell cycle arrest (1, 2). Given the high prevalence of genetic alterations in the *p53* gene found in tumors, *p53* is thought to play a role in the carcinogenic process of many forms of cancer. The reported frequency of alterations in the *p53* gene in colon tumors varies, with early reports suggesting that 75% of colon cancers had an alteration, although more recent estimates suggest that 40–50% of colon tumors have alterations (3–5). Most mutations in colon tumors have been reported as missense mutations, and studies that have examined the entire gene report that 87% of *p53* mutations in colon tumors occur in exons 5–8, the recognized regions that contain hot spots for *p53* mutations in human neoplasia (6). Over half of the mutations in colon tumors have been reported as occurring in one of three codons: 175, 248, and 273, and most are G:C to A:T transitions (5, 6).

The diet, lifestyle, environmental, or genetic factors that are associated with *p53* mutations in colon tumors are unknown; however, there is precedence for environmental factors affecting the type and/or location of *p53* mutations in other tumors. Hepatomas associated with aflatoxin B_1 and hepatitis B commonly show a mutation in codon 249 (6). Lung and esophageal cancer, diseases associated with tobacco usage, show frequent G:C to T:A transversions (6). Dietary components have been associated with specific Ki-*ras* mutations in colon tumors (7). Given the important role of diet and lifestyle factors to the etiology of colon cancer overall, it is a reasonable hypothesis that they are associated with p53 mutations and possibly with the location and type of mutation (8). In this study, we use data collected as part of a multicenter case-control study to evaluate how diet and other lifestyle factors relate to *p53* mutations in colon tumors.

Received 9/21/01; revised 3/6/02; accepted 3/26/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This study was funded by National Cancer Institute CA48998 and CA61757 (to M. L. S.), the Utah Cancer Registry, which is funded by Contract N01-PC-67000 from the National Cancer Institute, with additional support from the State of Utah Department of Health, the Northern California Cancer Registry, and the Sacramento Tumor Registry.
[2] To whom requests for reprints should be addressed, at Health Research Center, Department of Family and Preventive Medicine, 375 Chipeta Way, Suite A, Salt Lake City, UT 84108.

Materials and Methods

Study participants were African American, white, or Hispanic and were from either the KPMCP[3] of northern California, an eight county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties), or the Twin Cities metropolitan area in Minnesota (9). Eligibility criteria for cases included diagnosis with first-primary incident colon cancer (International Classification of Diseases for Oncology 2nd edition codes, 18.0 and 18.2–18.9) between October 1, 1991, and September 30, 1994, between 30 and 79 years of age at time of diagnosis, and mentally competent to complete the interview. Cases with adenocarcinoma or carcinoma of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening), with known familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were not eligible. Of all cases identified, 75.6% of those we were able to contact participated in the study, and 96% of cases identified were contacted.

Controls, in addition to the eligibility criteria for cases, could never have had a previous colorectal cancer. Controls were selected from eligibility lists from the KPMCP and driver's license lists in Minnesota, and from driver's license lists, random-digit-dialing, or Health Care Finance Administration lists for Utah. These methods have been described in detail (9, 10). Of all controls selected, 63.7% participated; the contact rate at KPMCP was 97%, in Utah it was 94%, and in Minnesota it was 72%.

Data Collection. Trained and certified interviewers collected diet and lifestyle data (11). The referent period for the study was the calendar year ~2 years before date of diagnosis (cases) or selection (controls). Respondents were asked to recall information on demographic factors: physical activity (12); body size, including usual adult height and weight 2 and 5 years before diagnosis; use of aspirin and/or NSAIDs; cigarette-smoking history; medical and reproductive history, including use of hormone replacement therapy; and diet.

Dietary intake data were ascertained using an adaptation of the validated Coronary Artery Risk Development in Young Adults diet history questionnaire (13, 14). Participants were asked to determine which foods were eaten (using brand names of food items such as fast foods, cookies, crackers, and cereals, when possible), the frequency with which foods were eaten, and the type of fat used in preparation of foods. Three-dimensional food models were used to help participants estimate their usual serving size. Cue cards were used to provide a consistent prompt to help identify foods within broad categories. For categories in which many types of food might have been eaten (such as breakfast cereal), participants were asked to report the three most commonly eaten items. Detailed information was also obtained on foods eaten as additions to other foods (such as sugar added to cereal); standard amounts of additions were assigned per unit of the food item they accompanied. Nutrients were calculated using the Minnesota Nutrition Coordinating Center's nutrient database, version 19; version 30 of the Minnesota Nutrition Coordinating Center's database was used to obtain data on *trans*-fatty acids. Foods were grouped into categories of red meat, processed meats (including hot dogs, luncheon meat, and sausage), eggs, low-fat dairy products (including milk, yogurt, and cheese), fruit (including fresh, frozen, and canned fruits), vegetables, cruciferous vegetables, whole grains, and refined grains. The number of standard servings consumed from a food group was totaled for each individual. A dietary glycemic index was created so that dietary carbohydrates could be weighed by their metabolic effect (15).

Tissue Ascertainment and DNA Extraction. Study respondents signed informed consent forms to have medical records, including tissue blocks, released. Detailed methods for obtaining tumor tissue have been discussed previously (16). Of the 2477 people for whom blocks were requested, DNA was extracted from 85.5%. Colon cancer tissue was microdissected, and DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks as described previously (16). Briefly, after preparation of H&E and aniline blue slides, the study pathologist (W. S.) reviewed the slides and selected material. Material was scraped into a 1.5-ml microfuge tube, combined with digestion buffer, and incubated overnight at 55°C. Samples were then heated to 94°C for 10 min to inactivate the proteinase K. Although DNA was available from more women than men, there were no detected differences among those with and without tumor DNA by age at diagnosis, tumor site, or interview status. Likewise, we detected no statistically significant differences in diet, BMI, physical activity, cigarette smoking status, or NSAID use for those with and without tumor DNA.

***p53* Analysis.** The exons and intron/exon boundaries of exons 5–8 of *p53* were PCR amplified with the following primers (two sets of primers, a 5′ and 3′ set, were used to amplify the relatively large exon 5; "F" is the forward primer, "R" the reverse): 5-5′F:ttatctgttcacttgtgccc and 5-5′R:tcatgtgctgtgactgcttg; 5-3′F:ttccacacccccgcccggca and 5-3′R:accctgggcaaccagccctg; 6F:acgacagggctggttgccca and 6R:ctcccagagaccccagttgc; 7F:ggcctcatcttgggcctgtg and 7R:cagtgtgcagggtggcaagt; and 8F:gtttctgcctcttgcttctctttt and 8R:tctcctccaccgcttcttgt. Primers were dye labeled and a SSCP analysis of the respective PCR products was performed by electrophoresis in a nondenaturing gel and evaluation on an ABI 373 machine (17). Tumor samples corresponding to any abnormally migrating SSCP bands were reamplified with the respective primers tailed with UP and RP (and without dye labeling) for sequencing. PCR products were sequenced using prism Big Dye terminators and cycle sequencing with Taq FS DNA polymerase. DNA sequence was collected and analyzed on an ABI prism 377-automated DNA sequencer. All alterations were verified by sequencing in both directions.

SSCP was also performed on the respective blood germline DNA sample of any tumor with a non-hot spot missense mutation, insertion or deletion mutations that consisted of multiples of 3 bases, and splice site mutations. For hot spot mutations in which the same mutation was found in ≥10 tumors, SSCP was performed on a random sample of 10 of the respective normal samples. If abnormally migrating SSCP bands were seen in any germ-line DNA sample, then these samples were also sequenced to determine whether the germ-line DNA harbored the same genetic alteration as the respective tumor. Sequencing also was performed on the corresponding germ-line DNA for any tumor in which multiple genetic alterations within a particular exon were identified by the initial sequencing. Any samples with a germ-line mutation were excluded as a tumor mutation.

Statistical Methods. Assessment of associations between diet and lifestyle factors and *p53* mutations in tumors were determined using multiple logistic regression models. Data were evaluated comparing those with *p53* mutations to those without

[3] The abbreviations used are: KPMCP, Kaiser Permanente Medical Care Program; NSAID, nonsteroidal anti-inflammatory drugs; BMI, body mass index; SSCP, single-strand conformational polymorphism; LSH, loop-sheet-helix; Wt, wild type; OR, odds ratio; CI, confidence interval; IHC, immunohistochemistry; IGF, insulin-like growth factor; IGF-IR, IGF-I receptor; GI, glycemic index.

Table 1 Description of *p53* mutations in the population

	Total *n* (%)	Men *n* (%)	Women *n* (%)	<65 *n* (%)	>65 *n* (%)
p53 Wt	772 (52.9)	409 (51.6)	363 (54.6)	321 (51.5)	451 (54.0)
p53 mutations	686 (47.1)[a]	384 (48.4)	302 (45.4)	302 (48.5)	384 (46.0)
Transition	528 (77.0)	287 (74.7)	241 (79.8)	240 (79.5)	288 (75.0)
Transversion	117 (17.1)	71 (18.5)	46 (15.2)	55 (18.2)	62 (16.1)
Missense	562 (81.9)	311 (81.0)	251 (83.1)	250 (82.8)	312 (81.3)
Frameshift	65 (9.5)	36 (9.4)	29 (9.6)	22 (7.3)	43 (11.2)
Stop	68 (9.9)	38 (9.9)	30 (9.9)	36 (11.9)	32 (8.3)
In-frame insertion/deletion	20 (2.9)	15 (3.9)	5 (1.7)	5 (1.7)	15 (3.9)
Splice site	18 (2.6)	10 (2.6)	8 (2.6)	10 (3.3)	8 (2.1)
CpG dinucleotides	355 (51.7)	201 (52.3)	154 (51.0)	163 (54.0)	192 (50.0)
R273	80 (11.7)	44 (11.5)	36 (11.9)	42 (13.9)	38 (9.9)
R248	79 (11.5)	39 (10.2)	40 (13.2)	30 (9.9)	49 (12.8)
L2 loop	110 (16.0)	66 (17.2)	44 (14.6)	54 (17.9)	56 (14.6)
L3 loop	143 (20.8)	74 (19.3)	69 (22.8)	63 (20.9)	80 (20.8)
LSH	189 (27.6)	102 (26.6)	87 (28.8)	86 (28.5)	103 (26.8)
β Sandwich	64 (9.3)	40 (10.4)	24 (7.9)	23 (7.6)	41 (10.7)
Contact	165 (24.1)	86 (22.4)	79 (26.2)	73 (24.2)	92 (24.0)
Structure	397 (57.9)	225 (58.6)	172 (57.0)	177 (58.6)	220 (57.3)
Denaturing	131 (19.1)	80 (20.8)	51 (16.9)	62 (20.5)	69 (18.0)

[a] The number of individuals with a *p53* mutation was 686. Because some people had multiple mutations, the total number of mutations does not add up to 686. Percentages are based on the percentage of people with the mutation rather than the percentage of all mutations.

p53 mutations, as well as those with and without *p53* mutations in tumors to population-based controls. The case-control comparison was conducted to estimate the relative risk of developing disease with specific genetic mutations. The "case-case" comparison was conducted to evaluate etiological heterogeneity of the risk factors under study.

In addition to evaluating any mutation, we evaluated the most common types of mutations, such as those occurring at CpG dinucleotides. We evaluated missense, transition, and transversion mutations as well as frameshift, in-frame insertions and deletions, stop codon, and splice site. The hot spots of Arg^{273} and Arg^{248} were evaluated separately because these were the most common hot spot mutations observed. We assessed functional impact of mutations by looking at contact mutations that inactivate *p53* by elimination of critical DNA contacts (Arg^{273}, Arg^{248}, Arg^{280}, and Cys^{277}) and structural mutations and noncontact missense mutations that appear to destabilize the structure of the core domain and location of mutation, including those located on the β sandwich, the L2 and L3 loops, and the LSH, including the helix (H2) portion of the protein (6, 17, 18). Specific types of mutations were assessed because other studies have shown specific mutations to have etiologic associations (6–8) and because missense mutations most closely correlate with IHC overexpression that has been done in other studies of associations (19).

Dietary data were analyzed for major dietary patterns found in the data set, as well as for major dietary components of those patterns (20). Dietary patterns were developed using factor analysis as described elsewhere using the SAS principal components program (20). After a varimax rotation, factor scores were saved for each individual. Two dietary patterns emerged that appeared to be important in this population. The food pattern arbitrarily labeled as "Western Diet" loaded heavily (factors with loadings of >0.30) on processed meats, red meat, fast-food meat, eggs, butter (men only), margarine, potatoes, high-fat dairy foods (men only), legumes, refined grains, added sugar (men only), sugar drinks (men only), and sugar desserts. The second dietary pattern, "Prudent Diet," loaded heavily on all types of fruits and vegetables, whole grains, fish, and poultry. Physical activity was assessed using an indicator of long-term vigorous physical activity (21). We also assessed the BMI of weight (kg)/height $(m)^2$, the use of aspirin and/or NSAIDs on a regular basis (defined as at least three times/week for 1 month), and the usual number of cigarettes smoked per day. Data were categorized into groups based on distribution in the control population.

Results

We detected *p53* mutations in tumors from 47.1% of the cases (Table 1); 48.4% of men and 45.4% of women had a tumor with a *p53* mutation. Among those 65 years of age and older, 46% had a *p53* mutation, whereas 48.5% of those younger than 65 years had a *p53* mutation. The majority of *p53* mutations were transitions (77%) rather than transversions (17.1%). Of specific types of mutations, 81.9% were missense, 9.5% were frameshift, 9.8% were stop codon, 2.9% were in-frame insertions or deletions, and 2.6% were splice site, and 51.7% of mutations were at CpG dinucleotides. Mutations designated as DNA contact mutations represented 23.7% of all mutations; structural mutations accounted for 58.7% of all mutations.

Compared with controls, there were no statistically significant age or gender differences for those with or without *p53* mutations (Table 2), although people with *p53* Wt were slightly older and more likely to be female than were people with a *p53* mutation. Never using aspirin or NSAIDs on a regular basis, smoking cigarettes, having a large BMI, and being more sedentary were traits reported more commonly by cases, both those with and without *p53* mutations, than by controls.

Colon cancer estimates of association with physical inactivity, BMI, not using aspirin/NSAIDs, and smoking were similar for those with and without a *p53* mutation compared with controls (Table 3). However, cases with a *p53* mutation were slightly more likely to consume a Western Diet compared with controls (OR, 2.03; 95% CI, 1.53–2.69) than were cases with *p53* Wt (OR, 1.57; 95% CI, 1.20–2.06). Specific components of the Western dietary pattern that were most strongly associated with *p53* specific disease pathway, as indicated by comparing

Table 2 Description of population by *p53* mutation status

	p53 mutation (n = 686) n (%)	*p53* Wt (n = 772) n (%)	Controls (n = 2410) n (%)
Age			
<50	58 (8.5)	61 (7.9)	229 (9.5)
51–64	244 (35.6)	260 (33.7)	760 (31.5)
65–69	132 (19.2)	142 (18.4)	489 (20.3)
70–79	252 (36.7)	309 (40.0)	932 (38.7)
χ^{2a}	0.24	0.28	
Gender			
Men	384 (56.9)	409 (53.0)	1290 (53.5)
Women	302 (44.0)	363 (47.0)	1120 (46.5)
χ^2	0.27	0.79	
Aspirin/NSAID (regular use)			
No	420 (61.2)	481 (62.3)	1260 (52.3)
Yes	266 (38.8)	291 (37.7)	1150 (47.7)
χ^2	<0.01	<0.01	
Cigarette smoking status			
Never	281 (41.2)	337 (43.8)	1121 (46.6)
<20 cigarettes/day	135 (19.8)	142 (18.5)	498 (20.7)
≥20 cigarettes/day	266 (39.0)	290 (37.7)	786 (32.7)
χ^2	<0.01	0.03	
BMI [wt (kg)/ht (m)^2]			
<25	235 (34.3)	252 (32.6)	955 (39.6)
25–29	217 (31.7)	281 (36.4)	831 (34.5)
>29	234 (34.2)	239 (31.0)	624 (25.9)
χ^2	<0.01	<0.01	
Long-term vigorous physical activity			
None	189 (27.6)	214 (27.7)	535 (22.2)
Intermediate	277 (40.4)	329 (42.6)	985 (40.9)
High	220 (32.1)	229 (29.7)	890 (36.9)
χ^2	<0.01	<0.01	

[a] χ^2 compares *p53* mutation to control and *p53* Wt to control.

cases with and without *p53* mutations, were diets that contributed to a high glycemic index and were high in sugar (OR, 1.50; 95% CI, 1.06–2.13) and diets high in red meat, fast food, and *trans*-fatty acids (OR, 1.38; 95% CI, 1.00–1.80; data not shown in table). Other dietary factors, including total energy intake, dietary folate, calcium, fiber, and specific foods were not associated with overall *p53* mutations. Additional adjustment for other diet and lifestyle factors did not alter the observed associations.

Gender and age-specific associations were observed for diet (data not shown in table). The high GI/sugar diet appeared to be associated with greater risk of a *p53* mutation compared to controls in women than in men (OR for women, 1.94; 95% CI, 1.23–3.06 and OR for men, 1.22; 95% CI, 0.83–1.77). Glycemic load appeared to be specifically associated with a p53 disease pathway for women (OR for female cases with *p53* mutations to *p53* Wt, 1.87; 95% CI, 1.10–3.17). The red meat/fast food/*trans*-fatty acid diet appeared to have a greater impact on developing a *p53* mutation among cases diagnosed at age 65 or older than among cases diagnosed after age 65 (OR comparing *p53* mutation to controls for <65 years, 1.66; 95% CI, 1.11–2.49 and OR for ≥65 years, 2.13; 95% CI, 1.49–3.05) than for *p53* Wt compared with controls (OR for <65 years, 1.40; 95% CI, 0.94–2.07 and OR for ≥65 years, 1.34; 95% CI, 0.95–2.38). Among older cases, the association with red meat/fast food/*trans*-fatty acid diet between those with and without a *p53* mutation was significantly different (OR, 1.59; 95% CI, 1.02–2.46).

Additional assessment comparing specific types of *p53* mutations to controls (Table 4) showed that Western dietary pattern overall and specific components of Western dietary pattern were associated with specific p53 mutations. Cases with transition and missense mutations were significantly more likely to eat a diet with a high glycemic load than cases without a *p53* mutation (OR transition mutation cases compared with case *p53* Wt, 1.77; 95% CI, 1.20–2.59 and OR for missense mutation compared with *p53* Wt, 1.72; 95% CI, 1.19–2.50). High levels of red meat/fast food/*trans*-fatty acid diet also were significantly associated with missense, transition, transversion, and CpG mutations. Cases who consumed a high red meat/fast food/*trans*-fatty acid diet with a missense *p53* mutation were significantly different from *p53* Wt cases (OR for missense, 1.40; 95% CI, 1.00–1.98). Physical inactivity, large BMI, and cigarette smoking showed slightly stronger associations with *p53* transversion mutations, although these differences were not statistically different from those detected in *p53* Wt cases. Missense and stop codon mutations were slightly more associated with not using NSAIDs when compared with controls, although these associations were not statistically different from those observed for *p53* Wt (Table 3).

Diets with a high glycemic load were more strongly associated with mutations in the L2 loop and at the Arg^{273} hot spot when compared with controls (Table 5) and compared with cases who were *p53* Wt (data not shown in table); high levels of these diets also were slightly more associated with mutations that influence protein structure. Diets high in red meat/fast food/*trans*-fatty acid were significantly associated with mutations that influenced protein structure when compared with controls (Table 5) or cases without *p53* mutations (OR cases with mutation in L2 loop compared with *p53* Wt, 2.44; 95% CI, 1.19–5.02 and OR cases with *p53* mutation in protein structure compared with *p53* Wt, 1.58; 95% CI, 1.07–2.32).

Discussion

Mutations of the *p53* tumor suppressor gene are some of the most commonly observed genetic alterations in human cancer (1–6). In this population-based study, we detected *p53* mutations in 47.1% of all colon tumors. This is comparable with other reports using similar methods to detect alterations (6, 19, 22, 23). As previously reported (6), the majority of mutations were missense in nature. Although few large-scale studies, and no population-based studies, have attempted, to date, to look at location of mutation on the gene, our data are comparable with those reports that exist. Our findings suggest that components of a Western-style diet are associated with *p53* mutations. Associations with a diet high with a high glycemic load were more pronounced among women than among men; associations with a diet high in red meat, fast food, and *trans*-fatty acid were more marked among people diagnosed when older than when younger.

Most epidemiological studies examining *p53* alterations in conjunction with diet and lifestyle factors have used IHC rather than sequencing to detect genetic alterations (19, 24). Most of these studies have had limited power. However, Voskuil *et al.* (19) studied 185 cases of colon cancer and 259 controls using both IHC and sequencing. IHC results showed 44% of cases with overexpression; sequencing results detected mutations in 32% of cases. Slightly stronger dietary associations were detected by IHC, although the strongest associations were detected among those without alterations in *p53*. In the study by Voskuil *et al.* (19), saturated fat appeared to have a greater influence on transversion mutations (OR, 2.0; 95% CI, 0.97–4.1 for interquartile range of intake) than other types of mutations.

Table 3 Associations[a] between diet and lifestyle factors in cases with and without *p53* mutations compared with controls

	OR (referent)	OR (95% CI)	OR (95% CI)	*P* trend
Diet				
Western	Low	Intermediate	High	
n cases M/Wt/controls	97/123/482	402/470/1461	187/179/467	
Mutation	1.00	1.37 (1.07–1.75)	2.03 (1.53–2.69)	<0.01
Wt	1.00	1.28 (1.02–1.60)	1.57 (1.20–2.06)	0.02
GI/Sugar	Low	Intermediate	High	
n cases M/Wt/controls	86/124/382	420/477/1490	180/171/538	
Mutation	1.00	1.25 (0.96–1.61)	1.48 (1.11–1.98)	<0.01
Wt	1.00	0.99 (0.79–1.24)	0.98 (0.75–1.28)	0.53
Red meat/fast food/*trans*-fatty acid	Low	Intermediate	High	
n cases M/Wt/controls	113/147/553	366/434/1356	207/191/521	
Mutation	1.00	1.28 (1.01–1.62)	1.92 (1.47–2.50)	<0.01
Wt	1.00	1.18 (0.95–1.46)	1.39 (1.08–1.80)	0.01
Lifestyle				
Physical activity	High	Intermediate	Low	
n cases M/Wt/controls	220/229/890	277/329/985	189/214/535	
Mutation	1.00	1.16 (0.95–1.42)	1.54 (1.22–1.93)	<0.01
Wt	1.00	1.31 (1.08–1.60)	1.59 (1.27–1.99)	<0.01
BMI [wt (kg)/ht $(m)^2$]	<25	25 –29	>29	
n cases M/Wt/controls	235/252/965	217/281/831	234/239/624	
Mutation	1.00	0.99 (0.80–1.24)	1.43 (1.16–1.76)	<0.01
Wt	1.00	1.24 (1.00–1.53)	1.49 (1.22–1.83)	<0.01
Smoking (cigarettes/day)	None	<20	≥20	
n cases M/Wt/controls	281/337/1121	135/142/498	266/290/786	
Mutation	1.00	1.08 (0.86–1.36)	1.33 (1.09–1.62)	<0.01
Wt	1.00	0.95 (0.76–1.19)	1.25 (1.04–1.51)	<0.01
Aspirin/NSAID (regular use)	Yes	No		
n cases M/Wt/controls	266/291/1150	420/481/1260		
Mutation	1.00	1.43 (1.20–1.71)		
Wt	1.00	1.53 (1.29–1.81)		

[a] Adjusted for age and gender; comparisons made to population-based controls. M, mutation.

Freedman *et al.* (24) found slightly stronger associations for most diet and lifestyle factors among those with *p53*+ tumors than those with *p53*− tumors when compared with controls in a study of 163 cases and 326 controls. In the study by Freedman *et al.* (24), tumors were considered *p53*+ when ≥20% cells were stained positive by IHC. Beef consumption was more strongly associated with *p53*− tumors when compared with controls than to *p53*+ tumors compared with controls; comparisons between *p53*+ and *p53*− tumors were not made (24). Using the same set of cases, Freedman *et al.* (25) also examined cigarette smoking and found that smoking cigarettes was associated with *p53* independent pathways because a much stronger association was observed when comparing *p53*+ cases to controls than when comparing *p53*− cases to controls. Zhang *et al.* (26), in a study of 107 patients with Dukes stage C colorectal cancer, used ≥25% positive cells to define a *p53*+ phenotype. They observed no differences in association between those who were *p53*+ *versus* those who were *p53*− for BMI, occupational activity, smoking cigarettes, drinking alcohol, or parity, although there were suggestions of a greater likelihood of having a *p53*+ tumor with bigger body weight.

These data represent the largest single data set of mutational analysis of colon tumors sequenced to date to our knowledge. However, because this is the first study to be able to evaluate types and location of mutations at this level of detail, these analyses can be considered exploratory and hypothesis generating. The majority of *p53* mutations occur in the core domain that contains the sequence-specific DNA binding activity of the *p53* protein (residues 102–292; 18). It is thought that the core domain is central to understanding how *p53* binds DNA and how tumorigenic mutations inactivate *p53*. The core domain structure consists of a β sandwich that acts as a scaffold for two large loops and a LSH. One class of mutations involves residues that contact the DNA; mutations in this area can be attributed to loss of critical DNA contacts that inactivate the *p53* gene. Other mutations appear to be critical for the stable folding of the core domain, and loss of DNA binding by these mutations can be attributed to structural defects. We observed that the Arg^{273} hot spot, a contact mutation located in the LSH motif, was influenced by a diet high in red meat, fast food, and *trans*-fatty acids. Other than individual hot spots, attempts have not been made to examine type or location of mutation in conjunction with diet and lifestyle factors. Associations appeared to be slightly stronger for mutations located on the L2 loop. Mutations located in specific areas of the gene may be important etiologically and may provide insight into different functional properties of the gene (8, 18).

Because of the size of the data set and genetic analyses by sequencing and SSCP, we have been able to evaluate diet and lifestyle associations with *p53* in more detail than has been done previously. However, for many factors, including physical activity, BMI, and using aspirin/NSAIDs, associations were similar for those with and without mutations, suggesting their association with many disease pathways. We observed the strongest associations for missense mutations, although, given that these types of mutations were more prevalent, we also had more power to detect significant associations. Associations with a Western dietary pattern were stronger among cases who had a *p53* mutation compared with controls than among cases who were *p53* Wt compared with controls. Certain components of a Western dietary pattern appear to account for the associ-

Table 4 Associations[a] between dietary and lifestyle factors and specific types of *p53* mutations compared with controls[b]

	OR (referent)	OR (95% CI)	OR (95% CI)	*P* trend
Diet				
Western	Low	Intermediate	High	
Transition	1.00	1.30 (1.00–1.71)	1.94 (1.43–2.65)	<0.01
Transversion	1.00	1.50 (0.85–2.65)	2.13 (1.13–4.03)	<0.01
Missense	1.00	1.31 (1.01–1.71)	1.92 (1.42–2.60)	<0.01
Frameshift	1.00	1.31 (0.67–2.56)	1.17 (0.50–2.77)	0.86
Stop codon	1.00	1.31 (0.63–2.74)	2.50 (1.13–5.54)	0.01
CpG	1.00	1.28 (0.93–1.76)	1.86 (1.29–2.68)	<0.01
GI/Sugar				
Transition	1.00	1.43 (1.06–1.93)	1.74 (1.25–2.43)	<0.01
Transversion	1.00	0.97 (0.57–1.65)	1.21 (0.67–2.20)	0.23
Missense	1.00	1.33 (1.00–1.77)	1.69 (1.23–2.33)	<0.01
Frameshift	1.00	0.91 (0.47–1.74)	0.53 (0.22–1.27)	0.20
Stop codon	1.00	1.23 (0.60–2.55)	1.25 (0.55–2.87)	0.96
CpG	1.00	1.37 (0.96–1.95)	1.54 (1.04–2.28)	0.05
Red meat/fast food/*trans*-fatty acid diet				
Transition	1.00	1.10 (0.85–1.42)	1.83 (1.37–2.45)	<0.01
Transversion	1.00	2.28 (1.23–4.25)	2.60 (1.31–5.16)	0.01
Missense	1.00	1.26 (0.98–1.63)	1.92 (1.14–2.56)	<0.01
Frameshift	1.00	1.16 (0.61–2.20)	1.29 (0.59–2.82)	0.54
Stop codon	1.00	0.97 (0.51–1.86)	1.60 (0.78–3.28)	0.10
CpG	1.00	0.92 (0.68–1.25)	1.57 (1.12–2.22)	<0.01
Lifestyle				
Physical activity	High	Intermediate	Low	
Transition	1.00	1.10 (0.88–1.38)	1.39 (1.08–1.80)	<0.01
Transversion	1.00	1.80 (1.13–2.87)	2.48 (1.47–4.19)	<0.01
Missense	1.00	1.22 (0.98–1.52)	1.59 (1.24–2.04)	<0.01
Frameshift	1.00	0.99 (0.55–1.78)	1.18 (0.61–2.30)	0.62
Stop codon	1.00	1.26 (0.72–2.23)	1.48 (0.76–2.89)	0.07
CpG	1.00	1.19 (0.91–1.55)	1.51 (1.11–2.05)	<0.01
BMI [wt (kg)/ht $(m)^2$]	<25	25–29	>29	
Transition	1.00	1.05 (0.83–1.32)	1.43 (1.13–1.80)	<0.01
Transversion	1.00	0.94 (0.58–1.54)	1.93 (1.24–3.01)	<0.01
Missense	1.00	0.98 (0.78–1.23)	1.52 (1.21–1.90)	<0.01
Frameshift	1.00	0.92 (0.50–1.69)	1.24 (0.68–2.27)	0.12
Stop codon	1.00	1.60 (0.90–2.86)	1.33 (0.70–2.52)	0.16
CpG	1.00	1.04 (0.79–1.37)	1.37 (1.04–1.81)	<0.01
Smoking (cigarettes/day)	None	<20	≥20	
Transition	1.00	0.95 (0.73–1.23)	1.27 (1.02–1.58)	<0.01
Transversion	1.00	1.32 (0.80–2.19)	1.60 (1.04–2.47)	<0.01
Missense	1.00	0.95 (0.74–1.23)	1.31 (1.06–1.62)	<0.01
Frameshift	1.00	1.49 (0.81–2.74)	0.99 (0.54–1.84)	0.70
Stop codon	1.00	1.40 (0.76–2.60)	1.24 (0.70–2.21)	0.14
CpG	1.00	0.85 (0.62–1.17)	1.17 (0.90–1.51)	0.14
Aspirin/NSAID (regular use)	Yes	No		
Transition	1.00	1.41 (1.16–1.71)		
Transversion	1.00	1.27 (0.87–1.85)		
Missense	1.00	1.37 (1.13–1.65)		
Frameshift	1.00	1.84 (1.09–3.11)		
Stop codon	1.00	2.12 (1.25–3.61)		
CpG	1.00	1.41 (1.12–1.78)		

[a] Adjusted for age and gender.
[b] For associations between Wt and controls, see Table 3.

ation with a *p53* disease pathway. These components, one representing high glycemic load and the other representing diets characterized by high intakes of red meat, fast food, and *trans*-fatty acids, imply that factors associated with the insulin and the IGF system levels may be associated with a *p53* disease pathway. Controlled studies suggest that Wt *p53* is necessary for the IGF-IR to function properly (27, 28). Studies have shown that sensitivity of tumor cells to circulating IGF-I is dependent on IGF-IR number; functional IGF-IRs are necessary for tumor formation and progression (29, 30).

This study advances our understanding of disease pathways and how diet and lifestyle factors are associated with specific pathways. Whereas *p53* is considered to occur later in the carcinogenic process, only 13.8% of tumors in this study had both a *p53* and Ki-*ras* mutation and 3.2% of tumors had both a *p53* mutation and microsatellite instability. In our previous work, we have shown that cigarette smoking and alcohol consumption are associated with microsatellite instability (31, 32). Dietary components also were associated with specific types of Ki-*ras* mutations (7): G→A mutations of the second base of codon 12 were associated with dietary factors hypothesized as being associated with DNA methylation, *i.e.*, folate,

Table 5 Dietary associations[a] between cases with *p53* mutations by location and functional definitions compared with controls

	OR (referent)	OR (95% CI)	OR (95% CI)	*P* trend
GI/Sugar	Low	Intermediate	High	
Location				
Arg^{273}	1.00	1.16 (0.58–2.32)	1.77 (0.84–3.73)	0.03
Arg^{248}	1.00	1.51 (0.74–3.10)	1.35 (0.60–3.07)	0.94
L2 loop	1.00	1.13 (0.61–2.09)	2.11 (1.11–4.01)	<0.01
L3 loop	1.00	1.49 (0.87–2.57)	1.51 (0.82–2.78)	0.88
LSH	1.00	1.13 (0.43–3.01)	1.48 (0.48–4.21)	0.10
Function				
DNA contact	1.00	1.31 (0.80–2.14)	1.53 (0.89–2.65)	0.16
Protein structure	1.00	1.36 (0.97–1.90)	1.78 (1.23–2.57)	<0.01
Denature	1.00	1.44 (0.82–2.52)	1.46 (0.78–2.74)	0.37
Red meat/fast food/ *trans*-fatty acid				
Location				
Arg^{273}	1.00	1.29 (0.69–2.42)	1.83 (0.90–3.70)	0.02
Arg^{248}	1.00	0.75 (0.43–1.31)	1.26 (0.67–2.39)	0.68
L2 loop	1.00	2.20 (1.15–4.22)	3.42 (1.70–6.86)	<0.01
L3 loop	1.00	0.93 (0.60–1.45)	1.54 (0.94–2.54)	0.26
LSH	1.00	2.19 (0.75–6.41)	2.93 (0.91–9.49)	0.06
Function				
DNA contact	1.00	0.95 (0.63–1.42)	1.44 (0.90–2.30)	0.08
Protein structure	1.00	1.47 (1.08–2.01)	2.19 (1.55–3.08)	<0.01
Denature	1.00	1.06 (0.66–1.69)	1.65 (0.97–2.80)	0.11

[a] Adjusted for age and gender.

vitamin B6, and vitamin B12; G→A mutations of the second base of codon 13 dietary factors were associated with insulin levels, *i.e.*, carbohydrates, refined grains, and glycemic index; and G→T mutations on the second base of codon 12 were associated with dietary fat, saturated fat, and monounsaturated fat. Additional exploration of interrelationship of diet and lifestyle factors and disease pathways is needed.

This study has several strong points, including the fact that it represents a population-based sample rather than a sample of cases that are derived from a limited number of clinics and/or hospitals. The dietary and other data were collected in a very rigorous manner. We attempted to collect tumor blocks on all cases identified in Utah and KPMCP and obtained blocks for the majority of cases in both centers (97% in Utah and 85% in KPMCP). Mutational status of the larger sample does not vary from the sample of interviewed cases. However, it is possible that despite rigorous laboratory techniques that included SSCP followed by sequencing detected variants that mutations could be missed. Likewise, because only the hot spots of the gene were sequenced it is possible that undetected mutations in other parts of the gene could dilute associations; however, studies have shown that almost 90% of all *p53* mutations in colon cancer are in these hot spots.

In summary, dietary components of a Western-style diet appear to contribute to a *p53*-colon cancer disease pathway. These components, one representing a diet associated with a high glycemic load and the other component representing a diet high in meat, fast food, and *trans*-fatty acids, appear to contribute most importantly to this disease pathway. These data suggest the importance of diet in relation to colon cancer and further suggest a specific disease pathway whereby these dietary factors operate.

Acknowledgments

We thank Margaret Robertson at the University of Utah DNA Sequencing Core Facility, Linda Ballard at the University of Utah Genomics Core Facility, Melanie Nichols and Kristen Gruenthal, and Dr. Mark Leppert for laboratory support. We also thank Dr. Bette Caan, Dr. John Potter, Judy Morse, and Leslie Palmer for their data collection efforts.

References

1. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K., and Vogelstein, B. Suppression of human colorectal carcinoma cell growth by wild-type p53. Science (Wash. DC), *249:* 912–915, 1990.

2. Chen, F., Chang, D., Goh, M., Klibanov, S. A., and Ljungman, M. Role of p53 in cell cycle regulation and apoptosis following exposure to proteasome inhibitors. Cell Growth Differ., *11:* 239–246, 2000.

3. Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Presinger, B. A., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., and Boss, J. L. Genetic alterations during colorectal tumor development. N. Engl. J. Med., *319:* 525–532, 1988.

4. Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Willson, J. K., Hamilton, S., and Vogelstein B. p53 mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res., *50:* 7717–7722, 1990.

5. Hollstein, M., Rice, K., Greenblatt, M. S., Soussi, T., Fuchs, R., Sorlie, T., Hovig, E., Smith-Sorenson, B., Montesano, R., and Harris, C. C. Database of *p53* gene somatic mutations in human tumors and cell lines. Nucleic Acids Res., *22:* 3551–3555, 1994.

6. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., *54:* 4855–4878, 1994.

7. Slattery, M. L., Curtin, K., Anderson, K., Ma, K. N. I., Edwards, S., Leppert, M., Potter, J., Schaffer, D., and Samowitz, W. S. Associations between dietary intake and Ki-ras mutations in colon tumors: a population-based study. Cancer Res., *60:* 6935–6941, 2000.

8. Walker, D. R., Bond, J. R., Tarone, R. E., Harris, C. C., Makalowski, W., Boguski, M. S., and Greenblatt, M. S. Evolutionary conservation and somatic mutation hotspot maps of p53: correlation with p53 protein structural and functional features. Oncogene, *18:* 211–218, 1999.

9. Slattery, M. L., Potter, J. D., Caan, B. J., Edwards, S. L., Coates, A., Berry, D. T., and Mori, M. Energy balance and colon cancer: beyond physical activity. Cancer Res., *57:* 75–80, 1997.

10. Slattery, M. L., Edwards, S., Caan, B., Kerber, R. A., and Potter, J. D. Response rates among control subjects in case-control studies. Ann. Epidemiol., *5:* 245–249, 1995.

11. Edwards, S., Slattery, M. L., Mori, M., Berry, T. D., and Palmer, P. Objective system for interviewer performance evaluation for use in epidemiologic studies. Am. J. Epidemiol., *140:* 1020–1028, 1994.

12. Slattery, M. L., and Jacobs, D. R., Jr. Assessment of ability to recall physical activity of several years ago. Ann. Epidemiol., *5:* 292–296, 1995.

13. Slattery, M. L., Caan, B., Duncan, D., Berry, T. D., Coates, A., and Kerber, R. A computerized diet history questionnaire for epidemiologic studies. J. Am. Diet. Assoc., *94:* 761–766, 1994.

14. Liu, K., Slattery, M. L., Jacobs, D. J., Cutter, G., McDonald, A., Van Horn, L., Hilner, J. E., Caan, B., Bragg, C., Dyer, A., and Havlik, R. A study of the reliability and comparative validity of the CARDIA dietary history. Ethn. Dis., *4:* 15–27, 1994.

15. Slattery, M. L, Benson, J., Berry, T. D., Duncan, D., Edwards, S. L., Caan, B. J., and Potter, J. D. Dietary sugar and colon cancer. Cancer Epidemiol. Biomark. Prev., *6:* 677–685, 1997.

16. Slattery, M. L., Edwards, S. L., Palmer, L., Curtin, K., Morse, J., Anderson, K., and Samowitz, W. Use of archival tissue in epidemiologic studies: collection procedures and assessment of potential sources of bias. Mutat. Res., *432:* 7–14, 2000.

17. Bonner, M. R., and Ballard, L. W. Considerations in adding mutation detection services to a sequencing core facility. J. Biomol. Tech., *10:* 177–186, 1999.

18. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. Crystal structure of a *p53* tumor suppressor-DNA complex: understanding tumorigenic mutations. Science (Wash. DC), *265:* 346–355, 1994.

19. Voskuil, D. W., Kampman, E., van Kraats, A. A., Balder, H. F., van Muijen, G. N. P., Goldrohm, R. A., and van't Veer, P. p53 overexpression and *p53* mutations in colon carcinomas: relation to dietary risk factors. Int. J. Cancer, *81:* 675–681, 1999.

20. Slattery, M. L., Boucher, K. M., Caan, B. J., Potter, J. D., and Ma, K. N. Eating patterns and colon cancer. Am. J. Epidemiol., *148:* 4–16, 1998.

21. Slattery, M. L., Edwards, S. L., Ma, K. N., Friedman, G. F., and Potter, J. D. Physical activity and colon cancer: a public health perspective. Ann. Epidemiol., *7:* 137–145, 1997.

22. Tortola, S., Marcuello, E., Gonzalez, I., Reyes, G., Arribas, R., Aiza, G., Sancho, F. J., Peinado, M. A., and Capella, G. p53 and K-*ras* gene mutations correlate with tumor aggressiveness but are not routine prognostic value in colorectal cancer. J. Clin. Oncol., *17:* 1375–1381, 1999.

23. Soong, R., Grieu, F., Robbins, P., Dix, B., Chen, D., Parsons, R., Houlse, A., and Iacopetta, B. p53 alterations are associated with improved prognosis in distal colonic carcinomas. Clin. Cancer Res., *3:* 1405–1411, 1997.

24. Freedman, A. N., Michalek, A. M., Marshall, J. R., Mettlin, C. J., Petrelli, N. J., Black, J. D., Zhang, Z. F., Satchidanand, S., and Asirwatham, J. E. Familial and nutritional risk factors for *p53* overexpression in colorectal cancer. Cancer Epidemiol. Biomark. Prev., *5:* 285–291, 1996.

25. Freedman, A. N., Michalek, A. M., Marshall, J. R., Mettlin, C., Petrelli, N. J., Zhang, Z. F., Black, J. D., Satchidanand, S., and Asirwatham, J. E. The relationship between smoking exposure and *p53* overexpression in colorectal cancer. Br. J. Cancer, *73:* 902–908, 1996.

26. Zhang, A. F., Zeng, Z. S., Sarkis, A. S., Klimstra, D. S., Charytonowicz, E., Pollack, D., Vena, J., Guillem, J., Marshall, J. R., Cordon-Cardo, C., Cohen, A. M., and Begg, C. B. Family history of cancer, body weight, and p53 nuclear overexpression in Duke's C colorectal cancer. Br. J. Cancer, *71:* 888–893, 1995.

27. Girnita, L., Girnita, A., Brodin, B., Xie, Y., Nilsson, G., Dricu, A., Lundeberg, J., Wejde, J., Bartolazzi, A., Winmna, K. G., and Larsson, O. Increased expression of insulin-like growth factor I receptor in malignant cells expression aberrant p53: functional impact. Cancer Res., *60:* 5278–5283, 2000.

28. Werner, H., Karnieli, E., Rauscher, F. J., and LeRoith, D. Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. Proc. Natl. Acad. Sci. USA, *93:* 8318–8323, 1996.

29. Butler, A. A., Yakar, S., Gewolb, I. H., Karas, M., Okubo, Y., and LeRoith, D. Insulin-like growth factor I receptor signal transduction: at the interface between physiology and cell biology. Comp. Biochem. Physiol. B Biochem. Mol. Biol., *121:* 19–26, 1998.

30. Butler, A. A., Blakesley, V. A., Poulaki, V., Tsokos, M., Wood, T. L., LeRoith, D., and Pouliki, V. Stimulation of tumor growth by recombinant human insulin-like growth factor I (IGF-I) is dependent on the dose and the level of IGF-I receptor expression. Cancer Res., *58:* 3021–3027, 1998.

31. Slattery, M. L., Curtin, K., Anderson, K., Ma, K. N., Ballard, L., Edwards, S., Leppert, M., Potter, J. D., Schaffer, D., and Samowitz, W. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J. Natl. Cancer Inst. (Bethesda), *92:* 1831–1836, 2000.

32. Slattery, M. L., Anderson, K., Curtin, K., Ma. K. N., Schaffer, D., and Samowitz, W. Dietary intake and microsatellite instability in colon tumors. Int. J. Cancer, *93:* 601–607, 2001.

Diet, Activity, and Lifestyle Associations with *p53* Mutations in Colon Tumors

Martha L. Slattery, Karen Curtin, K. Ma, et al.

Cancer Epidemiol Biomarkers Prev 2002;11:541-548.