Associations of mitochondrial polymorphisms with sporadic colorectal adenoma

Bharat Thyagarajan, University of Minnesota
Weihua Guan, University of Minnesota
Veronika Fedirko, Emory University
Helene Barcelo, University of Minnesota
Ramya Ramasubramaian, University of Minnesota
Myron Gross, University of Minnesota
Michael Goodman, Emory University
Robert M Bostick, Emory University

Journal Title: Molecular Carcinogenesis
Volume: Volume 57, Number 5
Publisher: Wiley: 12 months | 2018-05-01, Pages 598-605
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1002/mc.22783
Permanent URL: https://pid.emory.edu/ark:/25593/tqht4

Final published version: http://dx.doi.org/10.1002/mc.22783

Copyright information:
© 2018 Wiley Periodicals, Inc.

Accessed August 22, 2019 1:41 PM EDT
ASSOCIATIONS OF MITOCHONDRIAL POLYMORPHISMS WITH SPORADIC COLORECTAL ADENOMA

Bharat Thyagarajan1,5, Weihua Guan2, Veronika Fedirko3, Helene Barcelo1, Ramya Ramasubramaian4, Myron Gross1, Michael Goodman3, and Robert M. Bostick3

1Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, MN
2Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN
3Department of Epidemiology, Rollins School of Public Health, and the Winship Cancer Institute, Emory University, Atlanta, GA
4Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN

Abstract

Somatic mutations in mitochondrial DNA have been reported in colorectal adenomatous polyps (adenomas), the precursors to most colorectal cancers. However, there are no reports of associations of germline variation in mitochondrial DNA with adenoma risk. We investigated associations of germline polymorphisms in the displacement loop (D-loop) and non-D-loop region of the mitochondrial genome with incident, sporadic colorectal adenoma in three pooled colonoscopy-based case-control studies (n = 327 adenoma cases, 420 controls) that used identical methods for case and risk factor ascertainment. We sequenced a 1,124 bp fragment to identify all genetic variation in the mitochondrial D-loop region, and used the Sequenom platform to genotype 64 tagSNPs in the non-D-loop region. We used multivariable unconditional logistic regression to estimate associations of the polymorphisms with adenoma. The odds ratios (OR) for associations of four polymorphisms in the HV1 region (mt16294, mt16296, mt16278, mt16069) with adenoma were 2.30, 2.63, 3.34, and 0.56, respectively; all 95% confidence intervals (CI) excluded 1.0, however, after correction for multiple comparisons, none of the findings remained statistically significant. Similar results were found for six polymorphisms in the non-D-loop region. In the HV1 region poly C tract, relative to those with 5 repeats, the ORs for those with fewer or more repeats were, respectively, 2.29 (95% CI 1.07 – 4.89) and 0.63 (95% CI 0.36 – 1.08), but repeat numbers in the HV2 region were not associated with adenoma. These findings suggest that mitochondrial D-loop HV1 region polymorphisms may be associated with colorectal adenoma risk and support further investigation.

5Corresponding author: Dr. Bharat Thyagarajan, University of Minnesota, Department of Laboratory Medicine and Pathology, 515, Delaware Street SE, Room 1-136 Moos Tower, Minneapolis, MN 55455; Telephone Number: (612) 624-1257, thya0003@umn.edu.
INTRODUCTION

Mitochondria play an important role in energy metabolism and generate reactive oxygen species (ROS) that cause oxidative damage\cite{1,2}. ROS-induced oxidative damage plays an important role in early colorectal carcinogenesis starting even before the development of macroscopic colorectal adenomas, which are well recognized precursors for colorectal cancer\cite{3,4}. The human mitochondrial genome is a 16 kb circular double-stranded DNA molecule that encodes 13 polypeptide components of the electron transport chain (ETC), 22 tRNAs, and 2 rRNAs\cite{5,6}. The displacement loop (D-loop), the only non-coding region of the mitochondrial genome [nucleotide position (np) 16024-576 = 1124 bp], contains elements crucial for mitochondrial DNA (mtDNA) replication and transcription\cite{6,7}. The D-loop region contains two hypervariable regions (HV1: np 16024–16383 and HV2: np 57–333) that are mutational hotspots. In addition, both HV1 and HV2 regions contain poly C tracts that are important in regulating mtDNA replication\cite{7}. The mitochondrial genome has a higher mutation rate than does the nuclear genome due to the proximity of the mitochondrial genome to ROS production sites, limited DNA repair mechanisms in the mitochondria, and a lack of protective histone proteins\cite{8}. The D-loop region, in particular, accumulates mutations at a higher frequency than do other regions of the mitochondrial genome\cite{9}. Hence, sequence alterations in the D-loop region may contribute to altered replication and/or transcription of mitochondrial genes, which affect overall mitochondrial function and cellular ROS generation. Consistent with this observation, D-loop genetic variants were associated with mtDNA copy number\cite{10}, and somatic variants within the mitochondrial genome have been frequently identified in colorectal cancers\cite{11–13}. One study also reported associations of germline D-loop variants with colorectal cancer\cite{14}. Other studies reported associations of germline D-loop polymorphisms with risk of breast cancer\cite{15,16}, ovarian cancer\cite{17,18} and melanoma\cite{19}, and others reported associations of D-loop polymorphisms with hepatocellular carcinoma\cite{20} and ovarian cancer survival\cite{21}. In contrast to the previously reported association of D-loop polymorphisms with colorectal cancer\cite{14}, numerous previous studies found no associations of mitochondrial polymorphisms in the non-D loop region with the disease\cite{22–24}. Despite numerous reports of associations of mitochondrial polymorphisms with several cancers, including colorectal cancer, there are no reports of associations of mitochondrial variants with colorectal adenomas. Hence, we conducted a case-control study to (a) comprehensively evaluate associations of germline mitochondrial variants in the D- and non-D-loop regions with colorectal adenoma, and (b) assess associations of germline mitochondrial variants with mtDNA copy number.
MATERIALS AND METHODS

Study Population

The data for this analysis were pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma. The three studies, conducted between 1991 and 2002, used very similar recruitment and data collection protocols. Detailed descriptions of the three case-control studies, the Minnesota Cancer Prevention Research Unit (CPRU) study [25], the Markers of Adenomatous Polyps I (MAP I) study [26], and the Markers of Adenomatous Polyps II (MAP II) study [27], as well as the pooled study [28] were reported previously. Briefly, eligible participants between 30 and 74 years of age with no history of adenomatous polyps, familial polyposis, inflammatory bowel disease, bowel resection, or past or prevalent cancer other than non-melanoma skin cancer were scheduled for elective, outpatient colonoscopy in major gastroenterology clinics in the study locations. Cases had a pathologist-confirmed colorectal adenoma at colonoscopy, and controls had no polyps (adenomatous or hyperplastic) at colonoscopy. The consent rates in the CPRU, MAP I, and MAP II studies were 68%, 63.1%, and 76%, respectively. The total eligible population from all three studies included 797 cases and 1,000 controls (CPRU study: 574 cases and 707 controls; MAP I study: 174 cases and 177 controls; and MAP II study: 49 cases and 116 controls) [28]. Among these participants, DNA to perform the mitochondrial DNA copy number measurements and mitochondrial genotyping and D loop sequencing was available from 378 cases (47%) and 483 controls (48%). Participants with available DNA, relative to those with no DNA samples, on average, had a higher BMI (27.7 Kg/m$^2$ vs. 27.1 Kg/m$^2$; p=0.01), and were less likely to be never smokers (18.3% vs. 35.3%; p=0.003), more likely to regularly consume NSAIDs (22.8% vs. 19.4%; p=0.04), more likely to be black (4.3% vs. 2.4%; p=0.02), and more likely to have never consumed alcohol (12.9% vs. 10.9%; p=0.03).

Questionnaire Data Collection

Prior to their colonoscopy visits, participants completed mailed questionnaires regarding family history of cancer, medical history, diet (via semi-quantitative Willett food frequency questionnaires [29]), smoking and alcohol drinking habits, and self-measured anthropometrics. Information on adenomas identified during colonoscopy included the numbers, in vivo sizes, colon sites, histologic subtypes, and degrees of atypia. Fasting blood samples were obtained at the beginning of the colonoscopy visit. The protocols of each study were approved by the Institutional Review Boards of the corresponding institutions; the University of Minnesota and each Digestive Healthcare colonoscopy site for the CPRU study, Wake Forest University School of Medicine for the MAP I study, and the University of South Carolina for the MAP II study. All participants provided informed consent.

Laboratory Methods

D-loop region sequencing—The D-loop region of the mitochondrial DNA was amplified in two overlapping fragments, Fgt1 (mt14898 to mt151, 1.822 kb fragment) and Fgt2 (mt16488 to mt1677, 1.758 kb fragment), using the respective Fgt1 and Fgt2 primer sets.
The amplification reaction (12.5 μl) consisted of 2.5 ng of DNA, 0.5 U LongAmp Taq DNA polymerase (New England Biolabs, MA), 1X LongAmp buffer (New England Biolabs, MA), 400 uM of dNTP mix (New England Biolabs, MA), and 400 nM of each sequencing primer. The PCR cycle conditions were as follows: 1 cycle at 94°C for 3 mins., followed by 39 cycles at 94°C for 30 sec., 60°C for 30 sec., and 68°C for 60 sec., and then the reactions were held at 4°C if needed. The PCR product was purified prior to sequencing using the ExoSAP enzyme (Affymetrix, CA). The amplified mitochondrial fragments were submitted to the University of Minnesota Genomics Center (UMGC) for bi-directional Sanger sequencing by adding 3.2 pmol of the appropriate sequencing primer to 2 μl of the purified PCR product. The sequencing primers used for sequencing the Fgt1 and Fgt2 amplicons are listed below:

Fgt1 amplification primer (forward): mt14989: 5′ – TAGCCATGCACTACTCACCAGA - 3′
Fgt1 amplification primer (reverse): mt151: 5′ - GGATGAGGCAGGAATCAAAGAC - 3′
Fgt2 amplification primer (forward): mt16488: 5′ - CTGTATCCGACATCTGGTTCCT - 3′
Fgt2 amplification primer (reverse): mt1108: 5′ - TTTGGGGTTTGGTTGGTTCG - 3′

The sequences were assembled using Sequencher (Gene Codes Corporation, MI), and all of the polymorphisms were evaluated manually for accuracy.

**Non-D-loop region genotyping**—The 64 tagSNPs identified in a previous publication\[30\] were genotyped using the MassARRAY System combined with iPLEX chemistry (Agena Bioscience, CA) at UMGC.

**Mitochondrial DNA copy number**—Mitochondrial DNA copy number was estimated using a quantitative real time polymerase chain reaction (PCR) as described previously\[31\].

**Serum F₂-isoprostanes**—Serum F₂-isoprostanes concentrations were measured using GC-mass spectrometry\[32\]. An unweighted oxidative balance score that included a composite of anti- and pro-oxidant diet and lifestyle factors was calculated for each participant as described previously\[33\].
Statistical Analysis

Statistical analyses were carried out using SAS software version 9.1.3 (SAS Institute, Cary, NC). All P-values reported were two-sided. Selected characteristics of the cases and controls were summarized and compared using the $\chi^2$ test for categorical variables and the t-test for continuous variables. We used multivariable logistic regression to analyze associations of mitochondrial polymorphisms with colorectal adenoma after adjusting for the following covariates: age (continuous), sex, self-reported race, smoking status (never smokers vs. current or former smokers), regular (\textquotesingle once/week) intake of NSAIDs (yes/no), body mass index (BMI, continuous), and family history of cancer in a first degree relative (yes/no). Inclusion of other covariates such as regular aspirin intake, alcohol intake, physical activity, education, serum F$_2$-isoprostanes concentrations, and the oxidative balance score did not materially change the observed associations and were not included in the final models. The poly C tracts in the HV1 and HV2 regions were analyzed as categorical variables according to the length of the C repeats, based on the distribution of poly C repeats in the control population. The most common poly C repeat tract in the HV1 region (5 repeats) and in the HV2 region (7 repeats) were chosen as the reference groups for estimating associations of poly C tract repeat sizes with adenoma. For the associations of the mitochondrial polymorphisms with adenoma, we also investigated potential interactions of the mitochondrial polymorphisms (limited to those that were nominally associated \([p \leq 0.05]\) with colorectal adenoma) with circulating F$_2$-isoprostanes concentrations and the oxidative balance score.

To investigate associations of the mitochondrial polymorphisms with mtDNA copy number among the controls, we used multivariable linear regression, adjusting for age, race, sex, and study (CPRU, MAPI, MAPII). Among cases, we investigated associations of the D-loop and non-D-loop mitochondrial polymorphisms with adenoma multiplicity, colon site, size, subtype, and dysplasia using unconditional logistic regression models adjusted for age, sex, and race.

Since the poly C tract polymorphisms in the HV1 and HV2 regions were \textit{a priori} hypothesized to be associated with colorectal adenoma, a p-value $\leq 0.05$ for an association of any these polymorphisms with adenoma was considered to be statistically significant. Since there were no \textit{a priori} hypotheses for the other D-loop polymorphisms and the non-D-loop mitochondrial tagSNPs, we used Bonferroni correction to adjust for multiple comparisons (based on the number of SNPs with minor allele frequency $>5\%$ in the study population). Thus, a p value $\leq 0.002$ (0.05/30) was considered statistically significant for the D-loop polymorphisms; while a p value $\leq 0.003$ (0.05/16) was considered statistically significant for the non-D-loop tagSNPs.

RESULTS

Selected characteristics of the cases and controls are summarized in Table 1. Compared to the controls, the cases were, on average, older; more likely to be women, currently smoke, or currently consume alcohol; less likely to have a family history of colorectal cancer in a first degree relative or take non-steroidal anti-inflammatory medications regularly; and, on average, had a lower oxidative balance score.
The concordance among 25 blinded duplicates pairs was > 95% for the mitochondrial tagSNPs in the non-D-loop region and 100% for the D-loop polymorphisms. We identified 320 D-loop region genetic variants; most clustered in the hypervariable regions HV1 (n = 124; 39%) and HV2 (n = 111; 35%), and overall, only 30 mutations (9%) had a minor allele frequency (MAF) ≥5%. Four of the 64 tagSNPs in the non-D loop region were monomorphic. Sixteen tagSNPs (27%) had a minor allele frequency >5% and were included for further analysis.

After adjustment for covariates, three HV1 D-loop polymorphisms—mt16294, mt16296, and mt16278—were statistically significantly associated with 2.30-, 2.63-, and 3.34-fold higher risk for adenoma, respectively, and one (mt16069) was statistically significantly associated with approximately 44% lower risk; however, none of the findings for these variants remained statistically significant after adjustment for multiple comparisons (all Bonferroni-corrected p-values ≥0.002) (Table 2). In the non-D-loop region, three polymorphisms—mt709, mt930, and mt13708—were nominally associated with 1.75-, 2.42, and 1.93-fold higher risk, respectively, and three polymorphisms—mt13708, mt14798, and mt15073—were nominally associated with approximately 41%, 40%, and 51% lower risk, respectively; however, none of the findings for these variants remained statistically significant after adjustment for multiple comparisons (all Bonferroni-corrected p values > 0.003).

The distributions of the poly C tract polymorphisms in the HV1 and HV2 regions were similar to those in previously published studies\(^{10,14}\). In the HV1 region, the 5 C repeat polymorphism was observed in 85% of the study participants, repeat polymorphisms with ≤ 4 repeats (1–4 repeats) were found in 5%, and repeat polymorphisms with ≥ 6 repeats (6–13 repeats) were found in 10% of the participants (Table 2). In the HV2 region, the 7 C repeat was observed in 51% of the study participants, the 8 C polymorphism in 38%, and repeats sizes of ≥ 9 (9–15 repeats) were found in 11% of the participants (Table 2). The poly C tracts in the HV1 (16180–16195) and HV2 (303–317) regions were analyzed separately. Relative to individuals with 5 C repeats, those with ≤ 4 repeats were at statistically significant, 2.29-fold higher risk of adenoma, while those with ≥ 6 repeats were at an estimated 27% lower risk, although the finding was not statistically significant. In the poly C tract in the HV2 region, relative to those with the most common repeat polymorphism (7 Cs), neither those with 8 Cs nor those with ≥ 9 Cs were statistically significantly associated with adenoma (p ≥ 0.32).

Among the cases, none of the D-loop or non-D-loop mitochondrial SNPs associated with colorectal adenoma were associated with adenoma multiplicity, colon site, size, subtype, or dysplasia (data not shown).

Among the controls, of the polymorphisms that were nominally associated with colorectal adenoma were associated with adenoma multiplicity, colon site, size, subtype, or dysplasia (data not shown).
0.42 [95% CI 0.11–0.73] for the G allele; p=0.02). The poly C tract polymorphisms in HV1 (p=0.94) or HV2 regions (p=0.67) were not associated with mtDNA copy number. Further adjustment for mtDNA copy number did not substantially alter the estimated associations of mitochondrial polymorphisms with adenoma (data not shown). We found no evidence of multiplicative interactions of any of the selected mitochondrial SNPs (those that were nominally associated with adenoma) with circulating F$_2$-isoprostanes concentrations or the oxidative balance score in relation to the SNPs-adenoma associations (p ≥ 0.05 for all SNP-biomarker interactions).

**DISCUSSION**

This is the first study to report a) a comprehensive evaluation of associations of germline mitochondrial variations with colorectal adenoma risk, and b) an inverse association of germline repeat length polymorphism of the poly C tract in the HV1 region within the mitochondrial DNA D-loop region with colorectal adenoma. We found no evidence that the observed association was attenuated by adjustment for mtDNA copy number, or that circulating F$_2$-isoprostanes concentrations (an indicator of systemic oxidative stress) or the oxidative balance score (an indicator of the balance of anti- to pro-oxidant dietary and lifestyle exposures) interacted with the mitochondrial polymorphisms in relation to the repeat length polymorphisms-adenoma associations. Taken together, these findings suggest that the underlying mechanisms through which the poly C tract length polymorphism may influence colorectal adenoma risk are distinct from its roles in regulating oxidative damage or maintaining mtDNA copy number.

Two previous studies found somatic mitochondrial mutations in colorectal adenomatous polyps. One study of 40 colorectal cancers and 20 adenomas conducted in an Egyptian population found somatic mutations in both the D-loop and non-D-loop regions of the mitochondrial genome in 35% of adenomas and 25% of colorectal cancers[34]. Another study found somatic mitochondrial point mutations and instability in the poly C tract of the HV1 region in MUTFYH-associated polyposis, familial adenomatous polyposis (FAP), and sporadic adenomas, although mitochondrial mutations were more common in MUTFYH polyposis (9/11; 82%) than in FAP (6/13; 46%) or sporadic adenomas (4/19; 21%)[35]. In a retrospective cohort study of 365 patients from the Digestive Cancer Registry of Cote-d’Or (France), somatic D-loop mutations in colorectal tumors were associated with shorter colorectal cancer survival (3-year survival of 53.5% in patients with D-loop mutations vs. 62.1% in patients without D-loop mutations [p=0.05])[11]. However, this association with survival was not replicated in a subsequent study of 194 sporadic colorectal cancers in Taiwan[13]. In addition to somatic mitochondrial alterations, one case-control study of 174 colorectal cancer cases and 170 controls in South India reported that germline D-loop polymorphisms, such as the 310°C’ insertion, T16189C variant, and the 310°C’ins/16189C haplotype, were associated with colorectal cancer[14]. Our study extends the findings from these previous studies and is the first to report associations of germline polymorphisms in the mitochondrial D-loop region with colorectal adenomas. Specifically, we also found a longer poly C tract in the HV1 region to be inversely associated with colorectal adenoma. In addition, germline alterations (distinct from the poly C tract) of the HV1 region of the D-loop region were nominally associated with colorectal adenoma risk, though these estimated
associations were no longer statistically significant after adjustment for multiple comparisons. The T allele of the T16189C polymorphism results in an interrupted (i.e., shorter) poly C tract in the HV1 region, while the C allele codes for a longer, uninterrupted poly C tract. The shorter poly C tract in the HV1 region binds several proteins essential for mitochondrial replication more efficiently than the longer, uninterrupted poly C tract[36]. Similarly, the poly C tract in the HV2 region is also a replication primer binding site that is an important initiation site for mtDNA replication[37]. Hence, we hypothesized that longer, uninterrupted poly C tracts in the HV1 or HV2 regions may be associated with reduced mtDNA copy number. A previous cross-sectional study of 837 healthy adults reported an association of a longer poly C tract in the HV1 region with lower mtDNA copy number (mean mtDNA copy number of 2.47 in the uninterrupted poly C repeats (≥10 C) vs. 2.55 in the interrupted poly C tracts (4–9 poly C repeats); p=0.01)[10]. One previous study of breast cancer[38] found that heteroplasmy (more than one length of repeats) in the poly C tracts in the HV1 and HV2 regions in peripheral white blood cell mitochondrial DNA was associated with low mtDNA copy number (0.89 copies for the 5C repeat size in the HV1 region vs. 0.70 for other variants [p=0.03], and 0.88 for the 7C repeat size in the HV2 region vs. 0.72 for other repeat variants [p=0.03]). However, that study did not address whether variants had higher or lower repeat sizes[38]. In our study, the longer length poly C tract (≥6 C repeats) in the HV1 region was inversely associated with adenoma. However, we found no association of the poly C tract length polymorphisms in the HV1 or HV2 regions with mtDNA copy number. Instead, we found a single nucleotide substitution in the HV1 region (mt16296) to be inversely associated with mtDNA copy number. The biological mechanism(s) underlying this association remains unclear and needs to be investigated in future studies.

Three previous studies, in several populations, on various non-D-loop polymorphisms or mitochondrial haplogroups consistently found no associations of non-D-loop polymorphisms with colorectal cancer risk[22–24]. Thus, our findings of null associations of tagSNPs in the non-D-loop mitochondrial region with colorectal adenoma are consistent with those in previous studies of associations of mitochondrial haplogroups with risk of colorectal cancer. On the other hand, three common SNPs in the non-D-loop region, two SNPs (G752A and G1440A) in the 12S ribosomal RNA region, and one SNP (G4770A) in the nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2) region were associated with higher colorectal cancer mortality[39].

Our study has several limitations and strengths. The primary limitation was the relatively small sample size. Also, a relatively low proportion of participants from the three case-control studies had sufficient DNA to be included in these analyses, suggesting some limitations to the generalizability of our findings and raising the possibility of selection bias. However, the differences in the characteristics of those with and without DNA were modest, and, most importantly, the differences were not differential between the cases and the controls (data not shown), making selection bias unlikely. The participants in this study belonged to two racial/ethnic groups: European Americans and African Americans. Hence, the results of this study may not be generalizable beyond these racial/ethnic subgroups. Assessing diet via food frequency questionnaires has known limitations, such as recall error. The annual conversion rate from colorectal adenoma to colorectal cancer is only 0.25%, suggesting that an average adenoma-bearing individual is only at a moderate risk for
colorectal cancer [40]. Hence, associations of mitochondrial polymorphisms with colorectal adenoma do not necessarily imply an important role of mitochondrial polymorphisms in the development of colorectal cancer. Finally, because DNA from adenomatous tissue was not available in this study, we were unable to evaluate somatic mitochondrial alterations in the colorectal adenomas.

Strengths of our study include colonoscopic evaluation of both cases and controls and pathology-verification of cases, both of which reduce outcome misclassification. Also, detailed information was collected on covariates, which decreases unmeasured confounding, and questionnaires were administered prior to diagnosis, which reduces recall bias.

In conclusion, our results, taken in context with previous findings, suggest that the length polymorphisms within the HV1 region of the D-loop region of mitochondrial DNA may be associated with colorectal adenoma risk. Further studies are needed to investigate whether results similar to ours may be found in different study populations, including those in different racial/ethnic groups, and to evaluate potential biological mechanisms underlying our observed associations.

Acknowledgments

Research was supported by grant #s R03 CA167701, R01 CA116795, R01 CA066539, and P01 CA050305 from the National Cancer Institute (NCI).

References


Mol Carcinog. Author manuscript; available in PMC 2019 May 01.
**Table 1**

Selected characteristics of cases and controls in three pooled case-control studies of incident, sporadic colorectal adenomas, US, 1991–2002

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (N = 378)</th>
<th>Controls (N = 483)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study:</strong></td>
<td></td>
<td></td>
<td>0.0003</td>
</tr>
<tr>
<td>CPRU, %</td>
<td>66.7</td>
<td>57.8</td>
<td></td>
</tr>
<tr>
<td>MAPI, %</td>
<td>23.0</td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>MAPII, %</td>
<td>10.3</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>58.1 (9.0)</td>
<td>54.0 (10.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male, %</td>
<td>38.9</td>
<td>59.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>White, %</td>
<td>95.9</td>
<td>93.9</td>
<td>0.21</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.7 (5.4)</td>
<td>27.6 (5.6)</td>
<td>0.68</td>
</tr>
<tr>
<td>Currently smoke, %</td>
<td>23.4</td>
<td>14.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Family history of colorectal cancer **%, %</td>
<td>16.0</td>
<td>28.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Regularly take an NSAID ***, %</td>
<td>16.0</td>
<td>28.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Regularly take aspirin, % ***</td>
<td>33.0</td>
<td>35.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Physical activity, MET-hrs./wk.</td>
<td>219.6 (226.1)</td>
<td>220.5 (194.8)</td>
<td>0.95</td>
</tr>
<tr>
<td>Education:</td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>High school graduate, %</td>
<td>57.6</td>
<td>62.6</td>
<td></td>
</tr>
<tr>
<td>College graduate, %</td>
<td>31.0</td>
<td>30.1</td>
<td></td>
</tr>
<tr>
<td>Currently drink alcohol, %</td>
<td>8.8</td>
<td>16.1</td>
<td>0.006</td>
</tr>
<tr>
<td>Serum F_{2}-isoprostanes (pg/ml)</td>
<td>90.2 (51.3)</td>
<td>84.7 (36.5)</td>
<td>0.41</td>
</tr>
<tr>
<td>Oxidative Balance Score ****</td>
<td>−1.2 (4.8)</td>
<td>0.027 (5.3)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Abbreviations: CPRU, Cancer Prevention Research Unit; MAPI, Markers of Adenomatous Polyps; BMI, body mass index; NSAID, non-steroidal anti-inflammatory drug; MET, metabolic equivalent task; SD, standard deviation; CRC, colorectal cancer.

* Presented as mean (SD) except where otherwise indicated.

** In a first-degree relative.

*** At least once a week.

**** A composite of 15 anti- and pro-oxidant dietary and lifestyle exposures (see text); a higher score represents higher anti-oxidant relative to pro-oxidant dietary and lifestyle exposures.
Table 2

Associations of mitochondrial polymorphisms with colorectal adenoma in three pooled case-control studies of incident, sporadic colorectal adenomas, US, 1991–2002

<table>
<thead>
<tr>
<th>Mitochondrial variants</th>
<th>Cases (N = 378) (% Mutant)</th>
<th>Controls (N = 483) (% Mutant)</th>
<th>Odds ratio* [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-loop (HV1 region)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mt16294 C&gt;T</td>
<td>14.7</td>
<td>8.8</td>
<td>2.30 [1.38 – 3.73]</td>
<td>0.001</td>
</tr>
<tr>
<td>mt16296 C&gt;T</td>
<td>7.4</td>
<td>3.7</td>
<td>2.63 [1.30 – 5.30]</td>
<td>0.006</td>
</tr>
<tr>
<td>mt16278 C&gt;T</td>
<td>6.1</td>
<td>3.7</td>
<td>3.34 [1.26 – 8.86]</td>
<td>0.01</td>
</tr>
<tr>
<td>mt16069 C&gt;T</td>
<td>7.6</td>
<td>10.8</td>
<td>0.56 [0.33 – 0.96]</td>
<td>0.04</td>
</tr>
<tr>
<td>mt poly C tract: (HV1: 16180 – 16195)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 repeats</td>
<td>86.2</td>
<td>84.2</td>
<td>1.00 (REF)</td>
<td></td>
</tr>
<tr>
<td>≤4 repeats</td>
<td>6.4</td>
<td>3.7</td>
<td>2.29 [1.07 – 4.89]</td>
<td>0.03</td>
</tr>
<tr>
<td>≥6 repeats</td>
<td>7.4</td>
<td>12.1</td>
<td>0.63 [0.36 – 1.08]</td>
<td>0.10</td>
</tr>
<tr>
<td>mt poly C tract: (HV2: 303 – 315)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 repeats</td>
<td>52.1</td>
<td>49.4</td>
<td>1.00 (REF)</td>
<td></td>
</tr>
<tr>
<td>8 repeats</td>
<td>36.6</td>
<td>40.5</td>
<td>1.19 [0.85 – 1.69]</td>
<td>0.32</td>
</tr>
<tr>
<td>≥9 repeats</td>
<td>11.3</td>
<td>10.1</td>
<td>0.87 [0.50 – 1.49]</td>
<td>0.61</td>
</tr>
<tr>
<td>Non-D-loop region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mt709 G&gt;A</td>
<td>19.9</td>
<td>13.9</td>
<td>1.75 [1.17 – 2.61]</td>
<td>0.006</td>
</tr>
<tr>
<td>mt930 G&gt;A</td>
<td>7.5</td>
<td>3.8</td>
<td>2.42 [1.22 – 4.79]</td>
<td>0.01</td>
</tr>
<tr>
<td>mt11812 A&gt;G</td>
<td>9.7</td>
<td>5.5</td>
<td>1.93 [1.09 – 3.44]</td>
<td>0.02</td>
</tr>
<tr>
<td>mt13708 G&gt;A</td>
<td>9.2</td>
<td>13.3</td>
<td>0.59 [0.36 – 0.96]</td>
<td>0.04</td>
</tr>
<tr>
<td>mt14798 T&gt;C</td>
<td>10.3</td>
<td>14.7</td>
<td>0.60 [0.38 – 0.96]</td>
<td>0.04</td>
</tr>
<tr>
<td>mt15043 G&gt;A</td>
<td>3.49</td>
<td>5.89</td>
<td>0.49 [0.13 – 0.86]</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HV, hypervariable region

*From multivariable logistic regression; model covariates included: age (continuous), sex, self-reported race, smoking status (never smokers vs. current or former smokers), regular (≥ once/week) intake of NSAIDs (yes/no), body mass index (BMI, continuous), and family history of cancer in a first degree relative (yes/no).