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No Association between Mitochondrial DNA Copy Number and Colorectal Adenomas

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Abstract

Despite previously reported associations between peripheral blood mtDNA copy number and colorectal cancer, it remains unclear whether altered mtDNA copy number in peripheral blood is a risk factor for colorectal cancer or a biomarker for undiagnosed colorectal cancer. Though colorectal adenomas are well-recognized precursor lesions to colorectal cancer, no study has evaluated an association between mtDNA copy number and colorectal adenoma risk. Hence, we investigated an association between peripheral blood mtDNA copy number and incident, sporadic colorectal adenoma in 412 colorectal adenoma cases and 526 cancer-free controls pooled from three colonoscopy based case-control studies that used identical methods for case ascertainment, risk factor determination, and biospecimen collection. We also evaluated associations between relative mtDNA copy number and markers of oxidative stress, including circulating F₂-isoprostanes, carotenoids, and fluorescent oxidation products. We measured mtDNA copy number using a quantitative real time polymerase chain reaction (PCR). We used unconditional logistic regression to analyze the association between mtDNA copy number and colorectal adenoma risk after multivariable adjustment. We found no association between logarithmically transformed relative mtDNA copy number, analyzed as a continuous variable, and colorectal adenoma risk [odds ratio = 1.02, 95% CI: 0.82 – 1.27; p=0.86]. There were no statistically significant associations between relative mtDNA copy number and other markers of oxidative stress. Our findings, taken together with those from previous studies, suggest that relative mtDNA copy number in peripheral blood may more likely be a marker of early colorectal cancer than of risk for the disease or of *in vivo* oxidative stress.

Keywords

Mitochondrial DNA; Colorectal Adenoma; Oxidative Stress

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Introduction

Despite widespread use of colonoscopies for early detection and removal of colorectal adenomas, the precursors of most colorectal cancers, colorectal cancer remains the second leading cause of cancer death in the United States and is the only major cancer to affect both men and women essentially equally [1]. In addition, colorectal cancer remains the third most common cancer in the United States and accounts for 9% of all cancers with approximately 136,830 new cases expected to occur in 2014 [1].

Oxidative stress caused by reactive oxygen species (ROS) is thought to play an important role in colorectal carcinogenesis [2-10]. In addition, several studies identified oxidative stress as an important risk factor for colorectal adenomas, suggesting that abnormal oxidative stress may be important during early colorectal carcinogenesis. Mitochondria are both the major source and target of intracellular ROS [11,12], which are by-products of aerobic respiration. The amount of mitochondrial DNA (mtDNA) remains relatively stable within cells under physiological conditions [13,14]. Recent studies found that qualitative and quantitative mtDNA changes, such as somatic mitochondrial mutations in colorectal tumor tissue and alterations in mtDNA copy number may play a significant role in colorectal carcinogenesis [15,16]. Similarly, variations in mtDNA copy number in non-neoplastic tissues, such as peripheral blood, may reflect the net results of interactions between several unknown genetic and environmental factors that may increase oxidative stress and colorectal cancer risk. In support of this hypothesis, several studies found associations between mtDNA copy number in peripheral blood and a variety of cancers [17-28] and pre-malignant oral lesions [29]. In particular, a case-control study [24] and a prospective nested case-cohort study within the Singapore Chinese Health Cohort [25] both found mtDNA copy number in peripheral blood to be associated with increased colorectal cancer risk. Despite the prospective association between mtDNA copy number and colorectal cancer, it is unclear whether altered mtDNA copy number is a risk factor for colorectal cancer or a biomarker for undiagnosed colorectal cancer. Since colorectal adenomas are well-recognized precursor lesions to colorectal cancer, we hypothesized that mtDNA copy number would be directly associated with risk for colorectal adenoma, and thus support an etiological role for altered mtDNA copy number in colorectal cancer etiology. However, an association between mitochondrial DNA copy number and colorectal adenoma risk has not been previously evaluated. Therefore, we used data from three colonoscopy-based case-control studies to evaluate an association between peripheral blood mtDNA copy number and colorectal adenoma risk.

Material and Methods

Study Population

The participants for this study were pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma conducted between 1991 and 2002, with very similar recruitment and data collection protocols. Detailed descriptions of the designs and protocols used in the three case-control studies, the Minnesota Cancer Prevention Research Unit (CPRU) study [30], the Markers of Adenomatous Polyps I (MAP I) study [31], and the Markers of Adenomatous Polyps II (MAP II) study [32], as well as the pooled

study [33] were previously reported. Briefly, eligible participants between 30 and 74 years of age without any history of familial polyposis, inflammatory bowel disease, bowel resection, previous adenomatous polyps, incident colon cancer, 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 were those with no previously recorded or currently diagnosed colorectal adenoma. 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) [33]. Among these participants, sufficient DNA to perform the mitochondrial DNA copy number measurements was available from 412 cases (52%) and 526 controls (53%). Participants with available DNA were more likely to be alcohol non-drinkers (13.5% vs. 10.8%; $p=0.007$), black (4.6% vs. 2.2%; $p=0.001$), regular aspirin or non-steroidal anti-inflammatory drug (NSAIDs) users (49.7% vs. 42.7%; $p=0.0003$), and to have a slightly higher BMI (27.7 Kg/m² vs. 26.8 Kg/m²; $p<0.001$) than were participants without available DNA. There were no substantial differences in age, sex, family history of colorectal cancer, or smoking status between participants with and without DNA.

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 [34]), smoking and alcohol drinking habits, anthropometrics, and their reason(s) for colonoscopy. The numbers, *in vivo* sizes, colon sites, and histologic types of all polyps, and the subtype and degree of atypia for all adenomas were recorded. Blood samples were drawn at the time 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. Each participant provided informed consent.

Measurement of Mitochondrial DNA Copy Number

Blood was drawn into EDTA blood tubes and processed within 90 minutes to separate buffy coat and plasma. Buffy coat aliquots were stored at -70°C until DNA extraction. DNA was extracted from the blood samples and stored at -70°C until analysis. DNA for the CPRU study was extracted using the Flexigene DNA extraction kit (Qiagen Inc., Gaithersburg, MD, USA) at the University of Minnesota. DNA for the MAPI study was extracted from stored white blood cells digested in 500 µl of lysis buffer [50 mM Tris/HCl (pH 8.5), 1 mM EDTA, 0.2% SDS, and 200 g/ml proteinase K] overnight at 55°C with shaking. The digestion was precipitated directly with isopropanol, and the pellets were washed with 70% ethanol. The genomic DNA pellets (50–100 µg) were dissolved in 300–800 µl of TE [10 mM Tris (pH 8)-1 mM EDTA] buffer [31]. Genomic DNA for the MAPII study was extracted from 700 µl of whole blood on a Qiagen BioRobot M48 workstation employing a magnetic bead separation technology (Qiagen, Valencia, CA) following the manufacturer's instructions.

The details of the mtDNA copy number assay, which was conducted for all three studies at the same time in a central laboratory, were described previously [25]. Briefly, relative mtDNA copy number was measured using a real time quantitative polymerase chain reaction (PCR) using an Applied Biosystems 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). One primer pair specific for the mitochondrial DNA (*ND1*) and another primer pair specific for the nuclear DNA (*18s*) were designed for relative quantification for mtDNA copy number. Standard curves made by serial dilution of a reference DNA sample was used to determine the ratio of mtDNA copy number to the amount of nuclear DNA that is proportional to the mtDNA copy number in each cell. All samples were assayed in duplicate. A calibrator DNA (i.e., genomic DNA from a healthy control volunteer) was used to standardize analytical variation between different mtDNA copy number assay runs. The efficiency of all quantitative PCR runs ranged from 105% to 115%. The R^2 for all standard curves was 0.99. Standard deviations for the cycle of threshold (Ct) duplicates were 0.25. Based on analyses of 68 blinded duplicate samples analyzed on two different days, the coefficient of variation (CV) was 9.3%.

Biomarkers of Oxidative Stress

Due to the lack of sample availability in the CPRU study, F_2 -isoprostanes were measured only in the MAPI and MAPII studies. Fasting peripheral venous blood samples were drawn into red-coated, pre-chilled Vacutainer tubes and then immediately placed on ice and shielded from light. Blood fractions were aliquotted into amber-colored cryopreservation tubes, the air was displaced with an inert gas (nitrogen in MAPI and argon in MAPII), and then the aliquots were immediately placed in a -80°C freezer until analysis. We measured serum F_2 -isoprostanes using a method described previously [35]. The coefficient of variation (CV) for the serum F_2 -isoprostanes ranged from 9.5% to 11% among the control samples. We measured another recently utilized marker of oxidative stress, fluorescent oxidation products (FOP) using methods described previously [36]. The coefficient of variation (CV) for the FOP was 4.9% among the control samples. Serum carotenoids were measured using a HPLC-based method as described previously [37]. The coefficients of variation were less than 12.7% for all the serum carotenoids among the control pools. In addition, the oxidative balance score that combines pro- and antioxidant exposures using 15 different dietary and lifestyle exposures that are weighted equally [38] and was previously reported to be associated with incident sporadic colorectal adenoma was also evaluated as a composite indicator of oxidative stress.

Statistical Analysis

The relative mtDNA copy number was the ratio of the amount of mtDNA to nuclear DNA in the study sample normalized to a reference DNA sample. The distribution of mtDNA copy number was markedly skewed toward high values, which was normalized to a large extent by natural log transformation, and then the log transformed mtDNA copy number was analyzed as a continuous variable in the analysis. The χ^2 test and the t -test were used to compare the distributions of selected variables between colorectal adenoma cases and controls. Linear regression models were used to evaluate the association between relative mtDNA copy number and several demographic and lifestyle variables such as age, sex, race,

smoking status, family history of colorectal cancer, BMI, dietary factors such as total energy intake, fat intake, red meat and processed meat intake, and regular use of aspirin/NSAIDs among controls. Among cases, linear regression models were used to evaluate the association between relative mtDNA copy number and several adenoma characteristics such as number of adenomas, location of adenomas, size of the largest adenoma, and degree of atypia in the largest adenoma. Unconditional logistic regression models were used to evaluate the association between relative mtDNA copy number and colorectal adenoma risk after adjustment for age at sample collection (years), sex, race (Caucasian and non-Caucasian), regular aspirin or NSAID use, smoking status (never, former, and current), body mass index (BMI), and family history of colorectal cancer in a first degree relative (yes/no/unknown). Further adjustment for alcohol intake, physical activity, and dietary factors such as total energy intake and total intakes of red and processed meat, calcium, fruits and vegetables, and fiber did not materially change the estimated association between relative mtDNA copy number and colorectal adenoma and were not included in the final model.

We performed further analyses stratified by adenoma characteristics. Since the range of relative mtDNA copy number was different across the three case-control studies due to differences in DNA extraction techniques, we analyzed associations within each study, and then meta-analyzed the results from each of the three case-control studies using fixed effect models; from this, summary odds ratio (OR) estimates are presented. The heterogeneity *p*-value from the *Q*-test and I^2 statistic were also calculated to evaluate the degree of heterogeneity across the three studies. We performed linear regression analyses to evaluate the association between relative mtDNA copy number, oxidative stress-related markers (serum F_2 -isoprostanes, carotenoids [alpha carotene, beta carotene, lutein/zeaxanthin, beta cryptoxanthin and lycopene], and fluorescent oxidation products) and the oxidative balance score within each study after adjustment for the covariates listed above.

Statistical analyses were conducted using SAS software version 9.1.3 (SAS Institute, Cary, NC). The meta-analyses were performed using STATA software version 12 (metan package). All *P*-values reported are two-sided, and those that were less than 0.05 were considered to be statistically significant.

Results

Pooled analysis of all three case-control studies showed that participants with colorectal adenomas were, on average, older, more likely to be male, currently smoke, or consume alcohol; and less likely to have a family history of colorectal cancer in a first degree relative (Table 1). The mean relative mtDNA copy number among the controls was statistically significantly different across the three case control studies (CPRU vs. MAPI vs. MAPII, mean \pm SD: 0.75 ± 0.72 vs. -0.30 ± 0.58 vs. 1.40 ± 0.38 ; $p < 0.0001$). Therefore, we analyzed the association between mtDNA copy number and colorectal adenomas within each study individually and then combined the estimates using meta-analytical methods to provide a summary odds ratio. Among the controls, relative mtDNA copy number was not associated with age, sex, race, smoking status, family history of colorectal cancer, BMI, or regular use of aspirin/NSAIDs (data not shown). Among cases, a higher relative mtDNA copy number was associated with mild atypia in the largest adenoma as compared to moderate/severe

atypia in the largest adenoma (Summary OR: 1.17; 95% CI: 1.02-1.36; $p=0.03$, p for heterogeneity = 0.09) (Table 2). Relative mtDNA copy number was not associated with number of adenomas ($p=0.42$), adenoma location ($p=0.16$), or size of the largest adenoma ($p=0.47$) (Table 2). After adjustment for age, sex, race, smoking status, family history of colorectal cancer, BMI, and regular use of aspirin/NSAIDs, we found no association between relative mtDNA copy number and colorectal adenoma risk within each study or in all studies combined (per unit increase in log transformed relative mtDNA copy number, summary OR = 1.02, 95% CI: 0.82 – 1.27; $p=0.86$; p for heterogeneity = 0.43) (Table 3). Additional analysis stratified by sex, physical activity, obesity and aspirin/NSAIDs revealed no substantial or statistically significant associations between mtDNA copy number and colorectal adenomas in any of these subgroups (data not shown).

Though relative mtDNA copy number was statistically significantly higher among cases with moderate/severe atypia in the largest adenoma than among those with mild atypia in the largest adenoma, in the analysis stratified by degree of atypia, relative mtDNA copy number was not statistically significantly associated with adenoma risk among those with mild atypia (summary OR = 0.83, 95% CI: 0.63-1.09; $p=0.19$, p for heterogeneity =0.38) or moderate/severe atypia (summary OR = 1.24, 95% CI: 0.27-1.94; $p=0.12$, p for heterogeneity =0.08) (Table 3). There were also no substantial or statistically significant associations between relative mtDNA copy number and the other markers of oxidative stress; i.e., serum F₂-isoprostanes (β estimate (95% CI) = -0.001 (-0.004 - 0.002); $p=0.41$; p for heterogeneity = 0.50), serum fluorescent oxidation products (-0.347 (-0.739 - 0.040); $p=0.08$; p for heterogeneity = 0.12), and serum carotenoids ($p>0.43$; p for heterogeneity >0.002) or the oxidative balance score (0.006 (-0.006, 0.018); $p=0.32$; p for heterogeneity = 0.66) (Supplementary Figure 1).

Discussion

In our study, the first to investigate an association between mtDNA copy number and colorectal adenoma risk, we found no association between relative mtDNA copy number and other putative biomarkers of oxidative stress or with risk for incident sporadic colorectal adenomas.

Across several prospective studies, positive associations have been consistently found between relative mtDNA copy number and higher risk of a variety of cancers, such as non-Hodgkin lymphoma [18], and cancers of the lung [17], kidney [26], pancreas [21], breast [20], and colorectum [25]. Only one prospective study, of gastric cancer, found no association with mtDNA copy number [39]. The results from case-control studies have been mixed, with some studies reporting lower mtDNA copy number to be associated with higher risk of sarcomas [28] and renal [19,23] and esophageal cancers [27], while other studies reported a positive association between relative mtDNA copy number and risk of breast [22] and colorectal cancers [24]. In the above noted prospective study of colorectal cancer, a U-shaped association between relative mtDNA copy number and colorectal cancer risk was found, with both the lowest and highest two quartiles being associated with higher risk of colorectal cancer independent of the time interval between blood collection and colorectal cancer diagnosis [25]. In the present study, when we conducted similar analysis based on

quartiles of mtDNA copy number, we found no evidence of a U-shaped association between mtDNA copy number and colorectal adenoma risk (data not shown). In the previously reported case-control study of colorectal cancer, higher risk of colorectal cancer was found among participants with relative mtDNA copy numbers greater than the median level in the study population relative to those with numbers less than the median [24].

In our study we found large differences in relative mtDNA copy number among the three case-control studies. Samples from all three studies were randomly distributed across all analytical runs, and analysis of in-house laboratory controls revealed no evidence of laboratory drift/shift during the period in which these samples were analyzed. We previously found in our laboratory that differences in DNA extraction methods used across different laboratories can result in up to a 4-fold difference in relative mtDNA copy number (data not shown). Since DNA extractions for the three case-control studies were performed in different laboratories using different DNA extraction methods, this is the most likely explanation for the large differences in mtDNA copy numbers between the three studies. However, since DNA was extracted using a standardized extraction method within each study, differences in DNA extraction methods should not affect the observed associations between relative mtDNA copy number and colorectal adenoma risk within each study.

Though a previous prospective study found a direct association between relative mtDNA copy number and colorectal cancer risk [25], we found no association between relative mtDNA copy number and colorectal adenoma. Also, we found no association between relative mtDNA copy number and circulating levels of oxidative stress biomarkers or a food frequency questionnaire-based oxidative balance score. Furthermore, mtDNA copy number was not associated with other epidemiological factors that have been associated with increased oxidative stress, such as cigarette smoking or larger BMI. Despite mitochondria being a major source of ROS, we found no substantial evidence to support the hypothesis that relative mtDNA copy number in peripheral blood reflects global levels of oxidative stress. At least one previous study found that mtDNA copy number differs across the different white blood cell types (T lymphocytes, B lymphocytes, and monocytes) found in peripheral blood [40]. Hence, differences in mtDNA copy number between colorectal cancer cases and controls may reflect differences in cellular distributions between the two groups. A limitation common to our study and all previous studies that evaluated the mtDNA copy number-colorectal cancer/adenoma association is that since complete blood counts were not available, it was not possible to adjust for differences in distributions of various peripheral blood cell types. Furthermore, both previous studies that found an association between relative mtDNA copy number and colorectal cancer risk were conducted in Chinese populations, while the population for this study was predominantly Caucasian. Thus, a race-specific association of relative mtDNA copy number with colorectal adenoma/cancer risk cannot be ruled out. Finally, though colorectal adenomas are well-recognized precursors to most colorectal cancers, 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 of colorectal cancer [41]. Hence, a null association between relative mtDNA copy number and colorectal adenoma risk does not completely rule out an etiologic role for relative mtDNA copy number in colorectal carcinogenesis. Furthermore, we found that moderate/severe atypia in colorectal adenomas was associated with higher relative mtDNA

copy number, suggesting that colorectal adenomas may influence the amount of mtDNA copy number in peripheral blood.

In conclusion, the results of this study, taken together with those of previous studies, suggest that relative mtDNA copy number in peripheral blood may more likely be a marker of early colorectal cancer than of risk for the disease. Future studies aimed at elucidating the biological mechanisms underlying the observed associations between relative mtDNA copy number and colorectal cancer may help clarify a possible role of mitochondria in colorectal cancer etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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TABLE 1
Selected characteristics of cases and controls in three pooled case-control studies of incident, sporadic colorectal adenomas, US, 1991-2002

	Cases (n=412)	Controls (n=526)	P value
Study:			
CPRU, %	64.6	57.8	
MAPI, %	25.2	22.4	
MAPII, %	10.2	19.8	
Age, yrs., mean (SD)	58.2 (9.0)	53.9 (10.2)	<0.0001
Male, %	60	40	<0.0001
White, %	95.3	94.0	0.69
BMI, kg/m ² , mean (SD)	27.8 (5.4)	27.6 (5.7)	0.67
Physical activity, MET-mins./wk., mean (SD)	219 (226)	219 (195)	0.98
Education:			0.07
High school graduate, %	57.5	61.4	
College graduate, %	30.7	31.0	
Currently smoke, %	23.3	13.4	<0.0001
Alcohol non-drinkers, %	16.2	10.0	0.02
Family history of CRC, % *	16.9	29.2	<0.0001
Regularly take an NSAID, % **	16.6	27.8	<0.0001
Regularly take aspirin, % ***	32.9	35.1	0.49
Dietary intake per day			
Total energy, kcal	2,031 (775)	2,040 (747)	0.92
Red meats, servings	4.7 (3.6)	5.1 (6.3)	0.33
Processed meats, servings	2.6 (3.3)	2.4 (4.0)	0.47
Dietary fiber, g	21.1 (9.4)	21.8 (20.4)	0.53
Total *** calcium, mg	884.4 (492.6)	955.2 (743.2)	0.10
Serum F ₂ -isoprostanes (pg/ml)	92.5 (51.3)	85.2 (36.5)	0.23
Serum fluorescent oxidation products	0.059 (0.137)	0.052 (0.170)	0.52
Serum carotenoids (µg/dl)			

	Cases (n=412)	Controls (n=526)	P value
Alpha carotene	4.19 (3.40)	4.51 (4.59)	0.24
Beta carotene	14.46 (13.55)	16.04 (16.03)	0.12
Beta cryptoxanthin	6.11 (4.60)	6.69 (4.73)	0.07
Lutein/zeaxanthin	16.89 (7.36)	16.42 (8.00)	0.38
Lycopene	27.34 (13.18)	28.06 (13.58)	0.44
Oxidative Balance Score	-1.18 (4.75)	0.10 (5.26)	0.0001

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

* In a first-degree relative

** At least once a week

*** Dietary plus supplemental

Table 2
Associations of relative mitochondrial DNA copy number with adenoma characteristics among the cases (n = 405) in three case-control studies of incident, sporadic colorectal adenomas, US, 1991-2002*

Adenoma characteristics	Summary OR (95% CI); p value
Number of adenomas [‡]	0.97 (0.89 – 1.06); p = 0.54
Number of hyperplastic polyps [‡]	0.98 (0.94 – 1.03); p = 0.42
Size of largest adenoma [‡]	0.96 (0.85 – 1.08); p = 0.47
Degree of atypia in the largest adenoma	
<i>Mild atypia</i>	1.17 (1.02 – 1.36); p = 0.03
<i>Moderate/severe atypia</i>	1.0 (REF)
Location of adenomas	
<i>Cecum</i>	0.79 (0.56 – 1.12); p = 0.19
<i>Ascending colon</i>	1.03 (0.74 – 1.42); p = 0.88
<i>Hepatic flexure</i>	1.11 (0.75 – 1.63); p = 0.61
<i>Transverse colon</i>	0.93 (0.70 – 1.23); p = 0.61
<i>Splenic flexure</i>	1.32 (0.87 – 2.02); p = 0.19
<i>Descending colon</i>	0.79 (0.57 – 1.10); p = 0.16
<i>Sigmoid colon</i>	1.02 (0.83 – 1.25); p = 0.84
<i>Rectum</i>	1.0 (REF)

* Relative mtDNA copy number (dependent variable) was logarithmically transformed and analyzed as a continuous variable. All models were adjusted for age of the participant, sex, self reported race, regular intake of aspirin or other nonsteroidal anti-inflammatory drug (yes/no), family history of colorectal cancer in first degree relatives, body mass index, and smoking status (current vs. former vs. never) within each case-control study and then the meta-analyzed summary odds ratios across the 3 studies were calculated and presented.

[‡]Number of adenomas (range: 1 - 5), number of hyperplastic polyps (range: 0 - 9) and size of largest adenoma (range: 0.1 – 4 cm) were analyzed as continuous variables and the odds ratios represent change per unit change in these polyp characteristics.

Table 3
Association of relative mtDNA copy number with colorectal adenoma risk in three case-control studies of incident, sporadic colorectal adenomas, US, 1991-2002

	CPRU** OR (95% CI)	MAP1** OR (95% CI)	MAP11** OR (95% CI)	Summary OR (95% CI)	I ² (P _{heterogeneity})
Overall models*	0.98 (0.76 – 1.27)	1.28 (0.81 – 2.03)	0.69 (0.27 – 1.77)	1.02 (0.82-1.27)	0.0 (0.43)
Largest adenoma with mild atypia [†]	1.10 (0.79 – 1.53)	2.08 (1.19 – 3.63)	0.73 (0.27 – 1.94)	1.24 (0.27 – 1.94)	0.6 (0.08)
Largest adenoma with moderate/severe atypia [†]	0.90 (0.67 – 1.21)	0.51 (0.24 – 1.07)	0.72 (0.08 – 6.95)	0.83 (0.63 – 1.09)	0.0 (0.38)

* Relative mtDNA copy number was logarithmically transformed and analyzed as a continuous variable. The odds ratios represent effect sizes per unit increase in logarithmically transformed mtDNA copy number. All models were adjusted for age of the participant, sex, self reported race, regular intake of aspirin or other nonsteroidal anti-inflammatory drugs (yes/no), family history of colorectal cancer in first degree relatives, body mass index, and smoking status (current vs. former vs. never) within each case-control study and then meta-analyzed summary odds ratios across the 3 studies were calculated and presented.

[†]These models were adjusted for all the above-mentioned covariates in addition to degree of atypia in the largest adenoma (mild vs. moderate/severe vs. controls).

** Abbreviations: CPRU, Cancer Prevention Research Unit; MAP, Markers of Adenomatous Polyps; OR, odds ratio; CI confidence interval