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MITOCHONDRIAL COPY NUMBER IS ASSOCIATED WITH COLORECTAL CANCER RISK

Bharat Thyagarajan[1,5], **Renwei Wang**[2], **Helene Barcelo**[1], **Woon-Puay Koh**[3], and **Jian-Min Yuan**[2,4]

[1]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

[2]University of Pittsburgh Cancer Institute, Pittsburgh PA

[3]Saw Swee Hock School of Public Health, National University of Singapore, Singapore

[4]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA

Abstract

Background—Mitochondria are eukaryotic organelles responsible for energy production. Quantitative changes in human mitochondrial DNA (mtDNA) copy number have been implicated in various cancer types. Data from prospective cohort studies on mtDNA copy number and colorectal cancer risk have been lacking.

Methods—We evaluated the association between mtDNA copy number in peripheral blood and colorectal cancer risk in a nested case-control study of 422 colorectal cancer cases (168 cases with pre-diagnostic blood and 254 cases with post-diagnostic blood) and 874 controls who were free of colorectal cancer among participants of the Singapore Chinese Health Study. The relative mtDNA copy number was measured using real time PCR. Unconditional logistic regression methods were employed to examine the association between mtDNA copy number and colorectal cancer risk.

Results—There was a U-shaped relationship between the relative mtDNA copy number and colorectal cancer risk. Compared with the 2nd quartile, the odds ratios (95% confidence intervals) for subjects in the lowest and highest quartiles of relative mtDNA copy numbers were 1.81 (1.13–2.89) and 3.40 (2.15–5.36), respectively ($P_{curvilinearity}$ <0.0001). This U-shaped relationship was present in both men and women, similar for colon cancer and rectal cancer, and independent of the timing of blood draw with regards to cancer diagnosis.

Conclusions—This is the first prospectively designed study to show a U-shaped association between the relative mtDNA copy number and risk of colorectal cancer.

Impact—The findings of the present study support that mtDNA may play a critical role in the colorectal carcinogenesis in humans.

Keywords

Mitochondria; colorectal cancer; prospective; cohort; mitochondrial DNA copy number

[5]**Corresponding author:** Bharat Thyagarajan University of Minnesota Department of Laboratory Medicine and Pathology 515 Delaware Street SE, 1-136 Moos Towers Minneapolis, MN 55455 Phone: (612) 624-1257 Fax: (612) 624-8950 thya0003@umn.edu.

INTRODUCTION

Colorectal cancer is the fourth most common cancer in the United States and accounts for 9% of all cancers with approximately 141,210 new cases expected to occur in 2011 (1). Although early detection and removal of colorectal adenomas, the precursors of most colorectal cancers, has significantly reduced morbidity and mortality due to colorectal cancer, the malignancy 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, 2).

Oxidative stress caused by reactive oxygen species (ROS) is thought to play an important role in colorectal carcinogenesis (3–11). Mitochondria, whose principal function is to generate energy through aerobic respiration, are the major source and target of intracellular ROS (12, 13). Each cell has multiple copies of mitochondria and each mitochondrion has 2–10 copies of mitochondrial DNA (14, 15). The amount of mitochondrial DNA (mtDNA) remains relatively stable within the cells under physiological conditions (14, 16). Recent studies have found the alterations in mtDNA copy number and somatic mitochondrial mutations in colorectal tumor tissue suggesting that qualitative and quantitative changes of mtDNA may play a significant role in colorectal carcinogenesis in humans (17, 18). This hypothesis has been supported by some epidemiological studies that demonstrated a statistically significant association between increasing mtDNA copy number in peripheral blood and the increased risk of Non-Hodgkin lymphoma (19), lung cancer (20), pancreatic cancer (21), breast cancer (22), and colorectal cancer (23). However, other studies showed different results. One recent prospective study found no association between mtDNA copy number and gastric cancer (24) while a retrospective case-control study showed an increased risk of renal cancer associated with decreased mtDNA copy number (25). Given that the development of and treatment for cancer themselves can cause oxidative stress, which could results in the alteration of mtDNA copy number, a prospectively designed study with blood samples collected before cancer development could overcome this potential problem. Therefore we conducted a nested case-cohort study within the Singapore Chinese Health Study (SCHS) that blood samples were collected from the cohort participants before or after cancer diagnosis. The present study allowed us to evaluate the association between mtDNA copy number and the risk of colorectal cancer in pre-diagnostic as well as post-diagnostic peripheral blood samples.

MATERIAL AND METHODS

The study subjects were selected from the participants of the Singapore Chinese Health Study (SCHS), a population-based prospective investigation of diet and cancer risk. From April 1993 through December 1998, a total of 63,257 Chinese women and men aged 45–74 years were enrolled in the study (26). We restricted study subjects to the two major dialect groups of Chinese in Singapore - the Hokkiens who originated from the southern part of Fujian Province and the Cantonese who came from the central region of Guangdong Province. The subjects were residents of government housing estates; 86% of the Singapore population resided in these facilities. At recruitment, a face-to-face interview was conducted in the subject's home by a trained interviewer using a structured questionnaire that requested information on demographics, lifetime use of tobacco (cigarettes and water-pipe), current physical activity, menstrual/reproductive history (women only), occupational exposure, medical history, and family history of cancer. Information on current diet, including alcohol consumption, was assessed via a 165-item food frequency questionnaire that has been validated against a series of 24-hour dietary recall interviews (26). The Singapore Food Composition Table, developed in conjunction with this cohort study, allows for the computation of intake levels of roughly 100 nutritive and non-nutritive compounds per study subject (26).

In April 1994, one year after the initiation of the cohort study, blood and single-void urine specimens were requested from subjects drawn from a 3% random sample of enrollees. Participants who refused to donate blood were asked to donate buccal cells. The request for biospecimens was extended to all surviving members of the cohort beginning in January 2000. By April 2005, all surviving cohort subjects had been contacted for biospecimen donation. Biospecimens were obtained from more than 32,500 subjects. This study was approved by the Institutional Review Boards of the National University of Singapore, University of Pittsburgh, and the University of Minnesota.

Cases

We identified incident colorectal cancer cases through the population-based cancer registry in Singapore (27). The present study included cohort participants who donated a blood sample between April 1994 and July 1999 and developed colorectal cancer as of 31 December 2008. In total, 422 colorectal cancer patients constituted the case group of the present analysis. Histological and staging information on all colorectal cancer diagnoses were confirmed by manual review of the pathology reports and clinical charts. Two hundred and fifty-four patients donated their post-diagnostic blood samples (i.e., prevalent cancer case group) whereas the remaining 168 patients provided a blood sample before their development of colorectal cancer (i.e., incident cancer case group) of the present study.

Controls

Of the 3% random sample of study participants who donated a blood sample between April 1994 and July 1999 (n = 898?) 24 developed colorectal cancer by 31 December 2008 and were excluded from the control group. The remaining 874 subjects constituted the control group of the present study.

Laboratory Methods

Mitochondrial DNA copy number: Mitochondrial DNA (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). Two pairs of primers, one primer pair specific for the mtDNA (*ND1*) and another specific for the nuclear DNA (*18s*), were designed for relative quantification for mtDNA copy number. The ratio of mtDNA copy number to the amount of nuclear DNA was determined for each sample from standard curves made by serial dilution of a reference DNA sample. This ratio is proportional to the mtDNA copy number in each cell. The ratio for each sample was then normalized to a genomic DNA from a healthy control volunteer (calibrator DNA) to standardize analytical variation between different runs. The primer sequences for the mitochondrial ND1 gene were as follows: forward primer (ND1-F), 5′-CCCTAAAACCCGCCACATCT-3′; reverse primer (ND1-R), 5′-GAGCGATGGTGAGAGCTAAGGT-3′. The primer pair used for the amplification of the nuclear gene 18s was as follows: forward primer (18s-F), 5′-TAGAGGGACAAGTGGCGTTC-3′; reverse primer (18s-R), 5′-CGCTGAGCCAGTCAGTGT-3′. The PCR mixture in a total volume of 14 μL contained 1× SYBR Green mastermix (Applied Biosystems; Foster City, CA), 215 nM ND1-R (or 18s-R) primer, 215 nM ND1-F (or 18s-F) primer, and 4 ng of genomic DNA for *ND1* and 18s. The thermal cycling conditions were 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute for *ND1* and 62°C for 1 minute for *18s*. The efficiency of all quantitative PCR runs ranged from 99% to 110%. The R^2 for all standard curves was ≥ 0.99. Standard deviations for the cycle of threshold (Ct) duplicates were ≤ 0.25. All samples were assayed in duplicate on a 96-well plate. The coefficient of variation

(CV) was 17% based on analyses of 67 blinded duplicate samples analyzed on two different days.

STATISTICAL ANALYSES

The relative mtDNA copy number was the ratio of amount of mtDNA to nuclear DNA in the study sample normalized to a reference DNA sample. The distribution of mtDNA copy numbers was markedly skewed toward high values, which were corrected to a large extent by transformation to logarithmic values. Therefore, formal statistical testing was performed on logarithmically transformed values, and geometric (as opposed to arithmetic) means are presented. The χ^2 test and the *t*-test were used to compare the distributions of selected variables between colorectal cancer cases and controls. The analysis of covariance (ANCOVA) method was used to examine the difference in the relative mtDNA copy number across groups. These analyses were adjusted for age, gender and analytical run in which mtDNA copy number was measured (batch number) to control for variation in relative mtDNA copy number across different analytical runs. Unconditional logistic regression models were used to evaluate the association between relative mtDNA copy number and colorectal cancer risk after adjustment for age at sample collection (years), gender, body mass index (BMI), smoking status (never, ever), alcohol drinking (non-drinker, drinker), history of diabetes, physical activity and the batch number of mtDNA copy assays. The study participants were grouped into sex-specific quartile according to the distribution of relative mtDNA copy number among control subjects. To test for a curvilinear relationship between relative mtDNA copy number and colorectal cancer risk, we included a quadratic term, in addition to the linear term, for the relative mtDNA copy number. Sensitivity analysis was performed using unconditional logistic regression models stratified by timing of blood draw (pre-diagnostic vs. post-diagnostic samples), gender, cancer subsite (colon vs. rectum) or time interval between blood sample collection and colorectal cancer diagnosis among pre-diagnostic samples (≥ 3 years vs. < 3 years).

Statistical analyses were carried out using SAS software version 9.1.3 (SAS Institute, Cary, NC). All *P*-values reported are two-sided, and those that were less than 0.05 were considered to be statistically significant.

RESULTS

Participants with colorectal cancer were more likely to be older, men, smokers, less likely to have received high school education and having a history of diabetes as compared to controls (Table 1). The relative mtDNA copy number was significantly higher in women as compared to men (0.52 vs. 0.46; $p<0.0001$) (Table 2). The relative mtDNA copy number was also positively associated with dietary intake of α-carotene and β-carotene ($p\leq0.05$) (Table 2). Other factors such as age, history of diabetes, smoking status, alcohol intake, total energy intake, dietary intake of vegetables, BMI and moderate to vigorous weekly physical activity were not associated with relative mtDNA copy number (Table 2). Among prevalent cancer cases of colorectal cancer, there was no statistically significant difference in mtDNA copy number across different stages (Dukes A, B, C, and D staging) at diagnosis ($p=0.22$).

After adjustment for age at sample collection, gender, smoking, alcohol drinking, history of diabetes, BMI, physical activity and batch number, relative mtDNA copy number showed a U-shaped association with colorectal cancer risk. Compared with the 2nd quartile, participants in the lowest quartile and highest quartile of relative mtDNA copy number had an odds ratio (OR) of 1.81 (95% CI: 1.13–2.89) and 3.40 (95% CI: 2.15–5.36), respectively, for colorectal cancer, (p for curvilinear trend <0.0001) (Table 3). This U-shaped association

was similar for prevalent and incident colorectal cancer cases (both p for curvilinearity < 0.001) (Table 3).

We conducted additional analysis for the association between the relative mtDNA copy number and risk of incident colorectal cancer stratified by gender, cancer subsite and time interval between blood sample collection and colorectal cancer diagnosis. The U-shaped association was present in both men and women, for both colon and rectal cancers and among patients diagnosed with colorectal cancer ≥ 3 years or < 3 years after blood collection (Table 4). Compared with the 2nd quartile, OR for the lowest and highest quartile of the relative mtDNA copy number ranged from 2.81 to 5,23 and from 2.69 to 8.74, respectively, across different subgroups (all *Ps* for curvilinearity ≤ 0.03) However, the lowest quartile of the relative mtDNA copy number showed a greater OR for colorectal cancer risk in women (OR: 4.83, 95%CI: 1.42–16.38) than in men (OR: 3.72, 95% CI: 1.02–13.54), compared to the 2nd quartile. In contrast, the highest quartile of the relative mtDNA copy number was associated with a higher OR for colorectal cancer in men (OR: 8.74, 95%CI: 2.52–30.34) than in women (OR: 2.69, 95% CI: 0.75–9.69). However, the difference in the U-shaped relationship between men and women was not statistically significant (*P* for interaction = 0.97). The U-shaped association between the mtDNA copy number and risk of colon cancer was comparable with that between the mtDNA copy number and risk of rectal cancer (Table 4). The magnitude of U-shaped association between mtDNA copy number and cancer subsite (colon vs. rectum) and time interval between blood collection and colorectal cancer diagnosis (≥ 3 years vs. < 3 years) was comparable across all subgroups (Table 4).

DISCUSSION

This is the first study to prospectively evaluate the association between relative mtDNA copy number and colorectal cancer risk. The novel finding in this study is the U-shaped association between the relative mtDNA copy number in peripheral blood samples and colorectal cancer risk. Individuals with low and high relative mtDNA copy numbers are at increased risk of colorectal cancer.

The biological mechanism for the relative mtDNA copy number in peripheral blood and the risk of developing colorectal cancer is not completely understood. The mtDNA copy number in peripheral blood has been found to be correlated with the mtDNA copy number in normal colonic mucosa (r=0.66; p=0.04), suggesting that the mtDNA copy number in peripheral blood reflects for the mtDNA copy number in the target tissue (23). A positive association between the relative mtDNA copy number and markers of oxidative stress including thiobarbituric acid reactive substances and 8-hydroxyguanosine was observed in humans (28). Lower levels of antioxidants in plasma were associated with increased mtDNA copy number (28). Fibroblasts that were exposed to mild oxidative stress show an increase in mitochondrial mass through a cell-cycle independent pathway (29). High relative mtDNA copy number present in aging cells is thought to be the result of compensatory response to the cumulative exposures to oxidative stress and cumulative accumulation of mitochondrial DNA mutations over time (13). Multiple epidemiological studies with retrospective or prospective study design examined and found a positive association between mtDNA copy number and risk of cancers of various sites including the breast (21), colorectum (23), lung (20), pancreas (21) and NHL (19). Using the median mtDNA copy number among controls as the cutoff value, a retrospective case-control study of colorectal cancer showed higher mtDNA copy number as associated with 2.03 increased risk of colorectal cancer (95% CI: 1.41–2.81) (23). Reanalysis of the mtDNA copy number data of our prevalent cases from the present study using the median value among controls as the cutoff value showed results very similar to that previous retrospective case-control study (OR: 1.85; 95% CI: 1.30–2.64).

The present study also found that lower mtDNA copy number was associated with increased risk of colorectal cancer. An *in-vitro* study on HeLa cells showed that a reduction in mitochondria in HeLa cells resulted in increased lipid peroxidation and reduced antioxidant enzyme (e.g. catalase) activity (30). Furthermore, reduced mitochondria in HeLa cells also increased DNA damage in response to oxidative stress (30). More recently, mtDNA released from damaged mitochondria were shown to activate inflammasomes mediated by a member of the NOD-like receptor (NLR) family of cytoplasmic pattern recognition receptors, NLRP3. NLRP3 mediated inflammasomes cause the release of pro-inflammatory cytokines such as interleukin (IL)-1β and IL-18 (31). Consistent with the central role of inflammasomes in activation of inflammatory response, increased circulating levels of IL-18 have been associated with increased risk of colorectal and gastric cancers (32). The low number of mtDNA copy number may also reflect the reduced capacity of compensatory response of mitochondrial DNA to the damage caused by oxidative stress and environmental exposures. These data provide plausible biologic mechanisms by which the lower relative mtDNA copy number may be associated with increased colorectal cancer risk in the present study. Our findings of lower mtDNA copy number in relation to increased risk of colorectal cancer is consistent with a previous study showing that reduced mtDNA copy number was associated with increased risk of renal cancer (25).

Several studies examined mtDNA copy number in tumor tissues as well as adjacent normal tissues of various organs. Increased mtDNA copy number was observed in tumor tissues of the colorectum (17, 33), lung (33), ovary (34), endometrium (35) and thyroid (36) whereas reduced mtDNA copy number were observed in tumor tissues of the breast (36, 37), stomach (33, 38), liver (33, 39) and kidney (40), as compared to their normal adjacent tissues. These data suggest that the role of mtDNA in carcinogenesis might be different in different organs or tissue origin. Additional studies are warranted to elucidate the organ-specific role of mtDNA in the development of cancer in humans.

Our findings of high relative mtDNA copy number in women than in men is supported by a previous study (25). We also examined the association between lifestyle factors or history of diabetes and the relative mtDNA copy number among control subjects only. Individuals with high dietary intake of α- and β-carotenes had slightly higher relative mtDNA copy number. The significance of these findings remains unclear and these associations need to be confirmed in independent studies. The present study showed no statistically significant association for relative mtDNA copy number with body mass index, cigarette smoking, alcohol consumption, history of diabetes and physical activity. These are presumably oxidative stress factors. These results are consistent with those of some of previous studies (22–25), but different from others (25, 41).

The current study has several strengths. Singapore is a small city-state where there is good access to specialized medical care. The nationwide cancer registry has been in place since 1968 and has been shown to be comprehensive in its recording of cancer cases. Thus, colorectal cancer case ascertainment can be assumed to be complete. Our study subjects originated from two contiguous regions in South China, leading to a high degree of genetic homogeneity. All dietary and lifestyle factors which were included as covariates in the statistical models were assessed prior to cancer diagnosis and thus can be presumed to be free of recall bias. A relative large sample size of the present study provided sufficient statistical power to examine the association between the relative mtDNA copy number and colorectal cancer risk in all subjects as well as in subgroups that showed a robust U-shaped relation. It is unique that the present study included both prevalent and incident colorectal cancer cases. For prevalent cancer cases, blood samples were collected from patients after their cancer diagnosis and treatment, which resembled the situation in a typical retrospective case-control study. For incident cancer cases, blood samples were collected several years

prior to cancer diagnosis and treatment, which would avoid the potential impact of cancer progress and treatment on the relative mtDNA copy number in peripheral blood. The U-shaped association between the relative mtDNA copy number and risk of both prevalent and incident colorectal cancer suggests its important role in the colorectal cancer carcinogenesis.

A potential weakness of this study is the lack of repeated mtDNA copy number measurement in the same subject over time. To the best of our knowledge, there is no evidence that mtDNA copy number varies greatly over time for a given individual. We have conducted a small study and measured mtDNA copy number in peripheral blood from 5 volunteers every 2 weeks for a 2-month period. The within-individual coefficient of variation for mtDNA copy number over a 2-month period ranged from 7% to 24% (mean: 16%), which suggests that mtDNA copy number is relatively stable over time.

In summary, this is the first prospectively designed study to show a U-shaped association between the relative mtDNA copy number and risk of colorectal cancer. The robustness of the U-shaped association suggests its critical role in the colorectal carcinogenesis Future studies are warranted to evaluate ageing and oxidative stress related factors that influence mtDNA copy number and to provide novel insights into biological mechanisms of mtDNA copy number variation on the development of colorectal cancer.

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Table1

Baseline characteristics of cases and controls from the Singapore Chinese Health Study

Characteristics	Cases (n =422)	Controls (n = 874)	P value
Age at sample collection (yrs)	66.1 ± 8.0	57.6 ± 8.0	<0.0001
Gender (% male)	59.2	43.4	<0.0001
Dialect (% Cantonese)	45.3	50.6	0.07
Education (% secondary or high)	25.4	33.4	0.003
Smoking (% ever smoker)	42.9	26.1	<0.0001
Alcohol drinking (% weekly drinker)	23.7	19.5	0.08
History of diabetes (%)	14.2	9.5	0.01
Body Mass Index (BMI) (Kg/m^2)	23.0 ± 3.1	22.8 ± 3.1	0.12
Physical activity (% weekly)	35.1	35.4	0.92

Table 2

Geometric means of mtDNA by selected variables, among controls only

Variables	Number	Mean (95% CI)	P value
Age at sample collection *			0.11
45–50	201	0.50 (0.48–0.54)	
51–60	376	0.50 (0.46–0.52)	
61–70	224	0.48 (0.44–0.50)	
71+	73	0.48 (0.44–0.54)	
Sex **			<0.0001
Men	379	0.46 (0.44–0.48)	
Women	495	0.52 (0.50–0.54)	
BMI (Kg/m^2)			0.78
<20	147	0.50 (0.48–0.54)	
20–<24	484	0.48 (0.46–0.50)	
24–<28	202	0.50 (0.46–0.54)	
28+	41	0.48 (0.42–0.54)	
Smoking status †			0.10
Never	646	0.50 (0.48–0.52)	
Ever	228	0.46 (0.44–0.50)	
Alcohol drinking †			0.09
Non-drinkers	704	0.50 (0.48–0.52)	
Drinkers	170	0.46 (0.44–0.50)	
Diabetes †			0.08
No	791	0.50 (0.48–0.52)	
Yes	83	0.46 (0.40–0.50)	
Weekly physical activity †			0.92
No	565	0.48 (0.46–0.50)	
Yes	309	0.48 (0.46–0.52)	
α-carotene (mcg/day) †			0.03
1st quartile	192	0.46 (0.44–0.48)	
2nd quartile	217	0.48 (0.46–0.52)	
3rd quartile	227	0.50 (0.46–0.52)	
4th quartile	238	0.50 (0.48–0.54)	
β-carotene (mcg/day) †			0.05
1st quartile	202	0.46 (0.44–0.50)	
2nd quartile	207	0.48 (0.46–0.52)	
3rd quartile	217	0.52 (0.48–0.54)	
4th quartile	248	0.50 (0.46–0.52)	

Variables	Number	Mean (95% CI)	P value
Lutein (mcg/day) †			0.17
1st quartile	201	0.46 (0.44–0.50)	
2nd quartile	214	0.50 (0.46–0.54)	
3rd quartile	230	0.50 (0.46–0.52)	
4th quartile	229	0.50 (0.46–0.52)	
Green vegetable (g/day) †			0.14
1st quartile	199	0.46 (0.44–0.50)	
2nd quartile	212	0.48 (0.44–0.50)	
3rd quartile	241	0.52 (0.48–0.54)	
4th quartile	222	0.48 (0.46–0.52)	
Cruciferous vegetable (g/day) †			0.09
1st quartile	191	0.46 (0.44–0.50)	
2nd quartile	225	0.48 (0.46–0.52)	
3rd quartile	239	0.50 (0.46–0.52)	
4th quartile	219	0.50 (0.46–0.54)	
Yellow vegetable (g/day) †			0.07
1st quartile	208	0.48 (0.44–0.50)	
2nd quartile	212	0.48 (0.46–0.52)	
3rd quartile	221	0.48 (0.46–0.52)	
4th quartile	233	0.50 (0.48–0.54)	
Total energy intake (Kcal/day) †			0.40
1st quartile	190	0.48 (0.44–0.52)	
2nd quartile	229	0.48 (0.46–0.52)	
3rd quartile	231	0.50 (0.46–0.52)	
4th quartile	224	0.50 (0.46–0.52)	

*Adjusted for sex and batch number.

**Adjusted for age and batch number.

†Adjusted for age, sex, and batch number.

Table 3

Association between relative mtDNA copy number and colorectal cancer risk

mtDNA level*	No. of controls	All cases		Incident cases†		Prevalent cases†	
		n	OR‡ (95%CI)	n	OR‡ (95%CI)	n	OR‡ (95%CI)
1st quartile	219	130	1.81 (1.13–2.89)	61	3.51 (1.60–7.68)	69	1.32 (0.77–2.26)
2nd quartile	219	59	1.00	22	1.00	37	1.00
3rd quartile	219	74	1.40 (0.86–2.29)	27	1.58 (0.69–3.62)	47	1.20 (0.68–2.11)
4th quartile	217	159	3.40 (2.15–5.36)	58	4.66 (2.15–10.11)	101	3.37 (2.02–5.62)
P-value for curvilinearity			<0.0001		<0.0001		<0.0001

*Quartile levels are based on gender-specific cutoff values: men ≤ 0.36, 0.37–0.47, 0.48–0.62, >0.62; women ≤ 0.40, 0.41–0.54, 0.55–0.71, >0.71.

†Blood samples were collected 28.9 (SD = 26.4) months prior to cancer diagnosis for incident cases and 13.1(SD = 12.5) months after cancer diagnosis for prevalent cancer cases.

‡Adjusted for age at sample collection, gender, BMI, smoking status, alcohol drinking, weekly moderate or vigorous physical activity, history of diabetes and batch number for mtDNA copy number assay.

Table 4

mtDNA copy number in relation to the risk of colorectal cancer with pre-diagnostic blood samples by gender, colorectal cancer subsite, and duration between blood draw and cancer diagnosis

Subgroups		mtDNA copy number in quartile				P for curvilinearity
		1st quartile	2nd quartile	3rd quartile	4th quartile	
Men	Cases/Controls	28/95	12/95	11/95	41/94	
	OR* (95%CI)	3.72 (1.02–13.54)	1.00	2.10 (0.51–8.58)	8.74 (2.52–30.34)	0.005
Women	Cases/Controls	33/124	10/124	16/124	17/123	
	OR* (95%CI)	4.83 (1.42–16.38)	1.00	2.37 (0.65–8.58)	2.69 (0.75–9.69)	0.001
Colon cancer	Cases/Controls	44/219	13/219	16/219	29/217	
	OR† (95%CI)	3.36 (1.24–9.08)	1.00	1.42 (0.48–4.23)	2.94 (1.06–8.15)	0.001
Rectal cancer	Cases/Controls	17/219	9/219	11/219	29/219	
	OR† (95%CI)	3.17 (1.01–10.02)	1.00	1.39 (0.42–4.57)	7.64 (2.56–22.76)	<0.001
<3 years‡	Cases/Controls	48/219	16/219	16/219	41/217	
	OR† (95%CI)	2.81 (1.09–7.25)	1.00	1.08 (0.37–3.13)	5.27 (2.06–13.50)	<0.001
3+ years‡	Cases/Controls	13/219	6/219	11/219	17/217	
	OR† (95%CI)	5.23 (1.42–19.33)	1.00	2.65 (0.72–9.69)	3.77 (1.08–13.17)	0.03

*Adjusted for age at sample collection, BMI, smoking, alcohol drinking, diabetes, weekly moderate or vigorous physical activity, and batch number.

†Adjusted for age at sample collection, gender, BMI, smoking, alcohol drinking, diabetes, weekly moderate or vigorous physical activity, and batch number.

‡Number of years between blood draw and cancer diagnosis.