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Research Article

Cancer Prevention Research

Mitochondrial DNA Copy Number and Pancreatic Cancer in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study

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Abstract

Diabetes, obesity, and cigarette smoke, consistent risk factors for pancreatic cancer, are sources of oxidative stress in humans that could cause mitochondrial DNA (mtDNA) damage and increase mtDNA copy number. To test whether higher mtDNA copy number is associated with increased incident pancreatic cancer, we conducted a nested case–control study in the Alpha-Tocopherol Beta Carotene Cancer Prevention (ATBC) Study cohort of male smokers, aged 50 to 69 years at baseline. Between 1992 and 2004, 203 incident cases of pancreatic adenocarcinoma occurred (follow-up: 12 years) among participants, with whole blood samples used for mtDNA extraction. For these cases and 656 controls, we calculated ORs and 95% CIs using unconditional logistic regression, adjusting for age, smoking, and diabetes history. All statistical tests were two sided. Higher mtDNA copy number was significantly associated with increased pancreatic cancer risk (highest vs. lowest mtDNA copy number quintile, OR = 1.64, 95% CI = 1.01–2.67, continuous OR = 1.14, 95% CI 1.06–1.23), particularly for cases diagnosed during the first 7 years of follow-up (OR = 2.14, 95% CI = 1.16–3.96, P_{trend} = 0.01, continuous OR = 1.21, 95% CI = 1.10–1.33), but not for cases occurring during follow-up of 7 years or greater (OR = 1.14, 95% CI = 0.53–2.45, continuous OR = 1.05, 95% CI = 0.93–1.18). Our results support the hypothesis that mtDNA copy number is associated with pancreatic cancer and could possibly serve as a biomarker for pancreatic cancer development. *Cancer Prev Res; 4(11); 1912–9. ©2011 AACR.*

Introduction

Pancreatic adenocarcinoma is the fourth leading cause of cancer mortality in the United States (1) and the fifth worldwide (2). There is no effective screening method to detect early pancreatic cancer, which accounts for its poor 5-year survival rate of less than 5% (3). Although not well understood, mitochondria function and mitochondrial DNA (mtDNA) damage has been hypothesized to potentiate carcinogenesis (4). Mitochondria are both the primary target and the key intracellular source of reactive oxygen species (ROS) and oxidative stress (5). mtDNA is particularly susceptible to damage from ROS because they lack protective histones, have diminished DNA repair capacity, and are in close proximity to the electron transport chain, which releases ROS (6). Studies have suggested that mitochondria increase the number of copies of mtDNA to compensate for mtDNA damage and mitochondrial dysfunction (7). Each cell has thousands of mitochondrion, and each mitochondrion within normal cells have between 2 and 10 copies of their genomes, depending on the cell type (8). Previous studies investigating mtDNA copy number in leukocytes and disease outcomes have found an average mtDNA copy number in controls ranging from 96 to 107.3 (9, 10) and a log-transformed average mtDNA copy number for subjects older than 50 around 3.0 (11). Some of these studies included women only, women and men, and children; differences in mtDNA copy number by age or sex are not well understood.

Diabetes, obesity, and cigarette smoke, consistent risk factors for pancreatic cancer, are sources of oxidative stress in humans (12–15). Oxidative stress occurs when there is an imbalance between oxidants and antioxidants, and this imbalance could cause mitochondrial damage and affect mtDNA copy number. For instance, a component of cigarette smoke, polycyclic aromatic hydrocarbons (PAH), has a 40- to 90-fold greater affinity for

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mtDNA compared with nuclear DNA (16), and to compensate for damage from PAHs, mtDNA content increases (7). In a recent study, hyperglycemia was associated with increasing mtDNA content, and the mtDNA copy number was also correlated with plasma concentrations of thiobarbituric acid reactive substances, a biomarker of glucose metabolism (17). Because antioxidants found in food, vitamins and minerals (e.g. alpha-tocopherol, beta-carotene) help to neutralize ROS released during oxidative processes, we hypothesize that antioxidants also may be associated with reduced mtDNA copy number (18).

Although studies investigating mtDNA genomic mutations have been conducted in pancreatic cancer (19, 20), no study has evaluated the role of mtDNA copy number and pancreatic cancer risk. Therefore, to better elucidate the relationship between mtDNA copy number and pancreatic cancer, we conducted a nested case–control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study cohort of male smokers. The ATBC Study is uniquely positioned to investigate the relationship between mtDNA copy number and pancreatic cancer risk because of the exposure of population to oxidative stress due to smoking, their exposure to the beta-carotene and alpha-tocopherol study intervention, and the collection of biomarkers (beta-carotene, alpha-tocopherol, and retinol measured at baseline and after the study intervention), all of which can potentially modify mtDNA copy number.

Materials and Methods

Study population

The ATBC Study was a double-blind, placebo-controlled, 2×2 factorial-design primary prevention trial that tested whether alpha-tocopherol or beta-carotene supplementation reduced cancer incidence in Finnish male smokers (21). The study rationale, design, and methods have been described previously (21). Between 1985 and 1988, 29,133 eligible men aged 50 to 69 years in southwestern Finland who smoked at least 5 cigarettes per day were randomized to receive active supplements or placebo (21). Men were excluded from the study if they had a history of malignancy other than nonmelanoma skin cancer or carcinoma *in situ*, severe angina on exertion, chronic renal insufficiency, liver cirrhosis, chronic alcoholism, or another medical condition that might limit long-term participation; if they were receiving anticoagulant therapy; or if they were using supplements containing vitamin E (>20 mg/d), vitamin A (>20,000 IU/d), or beta-carotene (>6 mg/d). All study participants provided written informed consent prior to randomization, and the study protocol was approved by the Institutional Review Boards of both the National Public Health Institute in Finland and the National Cancer Institute in the United States.

During a prerandomization baseline visit, participants completed questionnaires on general background characteristics, including medical, smoking, dietary, and physical activity histories (21). Trained study staff measured height, weight, and blood pressure at baseline using standard methods. Body mass index (BMI; weight (kg)/height(m)^2) was calculated from measured weight and height. Diet was assessed with a validated self-administered dietary history questionnaire (21). Occupational activity was assessed by asking how much exercise and physical burden had been undertaken at work during the past year; responses could range from not working or sedentary to heavy physical work. Leisure-time activity was assessed by asking about the average level of activity engaged in during the past year; responses ranged from sedentary (reading, watching television) to moderate (walking, fishing, hunting, gardening regularly) to heavy or ''exercising to keep fit'' (running, jogging, or skiing regularly).

Serum samples were collected at 2 time points from study participants: (i) after an overnight fast, during the prerandomization visit on the entire cohort ($n = 29{,}111$); and (ii) 3 years after randomization at a follow-up visit on 26,546 study participants (postintervention). Whole blood samples were collected at one time point toward the end of the study between 1992 and 1993. The median time between baseline and the postintervention serum blood collection was 3.02 years; the median time between postintervention serum blood collection and whole blood collection was 2.65 years. All samples were stored at -70°C.

Case ascertainment and control selection

Cancer cases within the ATBC cohort were identified by linking the cohort to the Finnish Cancer Registry. The Finnish Cancer Registry provides nearly 100% case ascertainment in Finland and accurately reports 89% of primary pancreatic cancer cases (The ATBC Study, 1994; refs. 21, 22). We included incident primary malignant neoplasm of the exocrine pancreas (ICD9-157) and excluded endocrine tumors (ICD9-157.4) for this analysis. The pancreatic cancer diagnosis was confirmed through central review of all relevant hospital records for cases diagnosed from baseline through April 1999, whereas cases diagnosed after April 1999 were based solely on Finnish Cancer Registry data. Between 1992 and 2004 (follow-up to 12 years, median 6.3 years), 203 incident pancreatic cancer cases were identified among the subjects that had whole blood samples collected and from which DNA could be extracted.

Controls were selected from the ATBC Study and included participants who had whole blood samples for DNA extraction and were alive and free of cancer (except nonmelanoma skin cancer) at the time the case was diagnosed. Controls ($n = 406$) were matched to each pancreatic cancer case by date of birth (± 5 years) in a 2:1 ratio of controls to cases. We also included additional controls from other ATBC nested case–control studies ($n = 250$) with mtDNA data that were measured concurrently with our study measurements (23, 24). There were

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no statistically significant differences between the 2 control groups for the characteristics listed in Tables 1 and 2 (data not shown). Therefore, to increase study power, we used the pooled control set for this analysis ($n = 656$).

Biomarkers

Serum alpha-tocopherol and beta-carotene were measured within 2 years of serum collection by high-performance liquid chromatography as described in Milne and Botnen (25). DNA was extracted from peripheral white blood cells of the whole blood sample using the phenol–chloroform method and used to measure mtDNA copy number. Fluorescence-based quantitative PCR was used to determine the total mtDNA copy number by using the estimation of threshold cycle number of *ND1*, a mitochondrial gene, and that of the beta-globin gene, *HBB*, a nuclear gene (18). mtDNA copy number is reported as the number of mtDNA copies per cell.

Case and control specimens were handled in the same standard manner, and the laboratory was blinded to case–control status. Case and control samples were analyzed consecutively within batches, and blinded replicate "phantom" samples from 3 donor samples were placed within the batches, comprising 10% of each batch (26). Across all batches (pancreatic and lung cancers and lymphoma), the interbatch coefficient of variance (CV%) was 11.2% and the intrabatch CV% was 6.75%.

Statistical analysis

A total of 203 pancreatic cancer cases and 656 controls were used for this analysis. We compared distributions of selected case and control characteristics using the Wilcoxon rank-sum test for continuous variables and the χ^2 test for categorical variables (Table 1). Among control participants, generalized linear models were used to calculate mean values for continuous variables, and frequencies were used to calculate proportions (percentages) for the categorical variables to describe selected control characteristics across mtDNA copy number quintiles (Table 2). We also evaluated the correlation between continuous variables using Spearman correlation methods and results were similar to those reported in Table 2. Variables examined in the analyses and as potential confounders in the risk models were based on previous findings from the ATBC Study cohort or were associated with pancreatic cancer in the literature. Variables examined as potential confounders include those listed in Table 1, as well as self-reported history of chronic bronchial asthma, fruit and vegetable intake, and measured systolic and diastolic blood pressure. Dietary nutrients and foods highly correlated with energy intake were adjusted for energy using the residual method described by Willett and Stampfer (27).

Matching between cases and controls was not retained for the analyses because we used a pooled control set. Unconditional logistic regression was used to calculate ORs and 95% CIs for pancreatic cancer, with men in the lowest mtDNA copy number quintile serving as the reference category. mtDNA copy number was analyzed as both categorical and continuous variables. mtDNA was categorized into quintiles based on the distribution of controls. A score variable for mtDNA copy number based on the median values of each category was created to test trend. Continuous variables are presented based on a 21-unit change, which corresponds to the average unit change within quintiles 2 to 4 for the whole case–control set.

Multivariable models were developed using forward and backward regression by individually entering or subtracting potentially confounding variables into the model. mtDNA copy number was the exposure of interest and was therefore forced into all the models. Variables remained in the model if they were associated with both the disease and mtDNA copy number, had a *P* value of 0.10 or less in the full model, changed the risk estimate by more than 10%, or were putative risk factors for pancreatic cancer. The final multivariable models included putative risk factors for pancreatic cancer, namely age (continuous), smoking history (duration or years smoked and intensity or number of cigarettes per day-continuous), and history of diabetes (yes/no). Quartiles of mtDNA copy number were used in evaluating interactions to have adequate number of subjects in each cell. Effect modification of the mtDNA association by age, intervention, saturated fat intake, history of diabetes, BMI, smoking (cigarettes smoked per day and years smoked), and baseline and postintervention serum alpha-tocopherol and beta-carotene was evaluated in stratified analysis and by taking the cross-product terms of the mtDNA score variable, and the dichotomized effect modifier (with median split cutoffs) in multivariable models. We also stratified our analysis by follow-up time from whole blood collection to case diagnosis to assess the potential for reverse causation. We conducted sensitivity analyses, stratifying by follow-up time at 4, 5, 6, 7, 8, 9, and 10 years to determine whether risk changed during follow-up and found that 7 years best represented where the risk changed during follow-up. All statistical analyses were done using SAS software (SAS Institute Inc.). The *P* values for all statistical tests were 2-sided, and an alpha level of 0.05 was used to determine statistical significance.

Results

The median time from whole blood collection and pancreatic cancer diagnosis was 6.3 years. mtDNA copy number was significantly different between cases and controls ($P = 0.04$), with cases having a higher median mtDNA copy number (130.4; range 100–161.8 vs. 122.2; range 100–147.1; Table 1). Cases and controls did not significantly differ with respect to most characteristics; cases tended to have a greater history of diabetes, smoked for a longer time period, and consume more saturated fat and less carbohydrate (Table 1). The mean mtDNA copy number was 137.2 copies per cell for cases (SD, 50; range, 60.5–362.9) and 126.9 copies per cell for controls (SD, 37.3; range, 44.1–362.9).

Table 2 shows the means and percentages of selected baseline characteristics among the controls according to

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Table 1. Baseline characteristics of case and control subjects, ATBC study

Characteristics	Cases Median	(*n* = 203) IQR	Controls Median	(*n* = 656) IQR	*P*[b]
MtDNA copy number, median[a]	130.4	100.0–161.8	122.2	100.0–147.1	0.04
Age, y	58	54–61	58	54–62	0.78
Height, cm	174	170–179	173	169–178	0.14
BMI (units)	26.2	23.9–28.2	25.7	23.7–28.5	0.57
Smoking history					
Total cigarettes smoked/d	20	15–25	18	15–25	0.88
Years of smoking	37	31–42	36	31–42	0.08
Pack-years of smoking	35	24–45	34	23–44	0.13
Medical history (%)	7.4				
Diabetes mellitus	2		4.4		0.09
Pancreatitis	3.9		0.8		0.14
Gallstones	18.2		5.6		0.34
Peptic ulcers	75.4		17.1		0.70
Primary school education or less (%)	42.4		77.9		0.45
Living in city (%)	7.4		47.7		0.18
Daily dietary intake[c]					
Energy, kcal	2,688	2,236–3,170	2,585	2,164–3,136	0.25
Total fat, g[c]	123	114.3–133	122	113–132	0.28
Saturated fat, g[c]	53	45–64	52	43–60	0.07
Carbohydrate, g[c]	264	236–288	269	246–289	0.10
Protein, g[c]	94	87–100	94.1	86–101	0.79
Folate, μg[c]	337	275–402	328	269–392	0.37
Alcohol, g	9.2	2.42–26.8	10.7	2.5–24.0	0.96
Physical activity (%)					
Occupational activity					0.91
Sedentary	15.3		13.4		
Moderate	33		32.9		
Heavy	7.9		8.7		
Not working	43.8		45		
Leisure activity					0.49
Sedentary	40.1		36		
Light or moderate	53		55.3		
Exercising to keep fit	6.9		8.7		
Serum levels–baseline[c]					
Baseline AT, μg/L	11.9	9.7–13.8	11.8	10.0–13.6	0.65
Baseline BC, μg/L	162	110.0–270	190	122.0–281	0.17
Retinol, μg/L	574	479.0–664	574	496.0–668	0.53
Serum–postintervention[c]					
AT, μg/L[d]	17.2	14.9–20.8	17.6	15.2–20.1	0.98
BC, μg/L[d]	3,016	1,386–4,817	3117	2,124–3,907	0.87
Retinol, μg/L	596	494–674	584	503–683	0.72
Randomization group (%)					
Alpha-Tocopherol (AT)	24.6		25.6		0.87
Beta-Carotene (BC)	21.7		24.9		
AT and BC	26.1		24.2		
Placebo	27.6		25.3		

[a]Units of measurement = number of copies of mtDNA per cell.
[b]*P* values for categorical variables were based on the χ^2 test or Fisher's exact test; *P* values for continuous variables were based on Wilcoxon's rank-sum test.
[c]Adjusted by total energy intake. Number of controls = 609.
[d]Number of controls = 642; Postintervention serum measures limited to subjects in the intervention arm only.

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Table 2. Select characteristics of control subjects by mtDNA copy number (quintiles)[a]

	MtDNA copy number quintiles				
Characteristic	**Q1 ≤94.6**	**Q2 >94.6–≤113.3**	**Q3 >113.3–≤132.8**	**Q4 >132.8–≤158.0**	**Q5 >158.0**
mtDNA copy number[b,c]	83.2	104.2	123.6	144.5	186.2
Age, y[c]	58	58	58	58	58
Height, cm[c]	174	173	174	173	173
Body Mass Index, kg/m^2	26.4	26.5	26.1	26.4	26
Smoking history[c]					
Cigarettes smoked per day	20.5	18.8	19.3	18.8	18
Years of smoking	36.4	37	35.9	35.5	35.1
Pack-years of smoking	38.2	34.9	35	34.1	32.4
Medical history, %					
Diabetes mellitus	3.8	6.8	5.6	3.1	2.5
Pancreatitis	2.3	0	0	0.8	0.9
Gallstones	10.5	7.5	2.8	1.5	5.9
Peptic ulcers	20.3	15	16.2	15.4	18.6
Primary education or less, %	79	75.9	81	75.4	78
Living in city, %	45.9	46.6	51.4	40.8	54.2
Daily dietary intake[c]					
Energy, kcal	2,610	2,713	2,659	2,662	2,700
Total fat, g[d]	124	121	121	122	125
Saturated fat, g[d]	53	51	52	53	54
Carbohydrate, g[d]	268	266	272	269	270
Protein, g[d]	94	95	95	96	94
Folate[d], μg	333	348	342	336	344
Alcohol, g	15.8	20.2	16.5	16.3	13.8
Physical activity, %					
Occupational activity					
Sedentary	10.5	16.5	9.2	18.5	12.7
Moderate	36.8	35.3	30.3	28.5	33.9
Heavy	7.5	7.2	6.6	5.6	6.1
Not working	45.9	41.4	51.4	43.1	42.4
Leisure activity					
Sedentary	37.6	39.1	27.5	41.5	34.8
Light or moderate	53.4	54.9	60.6	51.5	55.9
Exercising to keep fit	9	6	12	6.9	9.3
Serum levels–baseline[c]					
AT, μg/L	12.1	12.4	12.1	12.2	11.9
BC, μg/L	230	214	228	233	230
Retinol, μg/L	565	602	583	607	588
Serum–postintervention[c,e]					
AT, μg/L	18.1	17.7	17.6	18.4	18.2
BC, μg/L	2,953	3,167	3,615	2,961	3,043
Retinol, μg/L	588	611	585	630	597
Randomization group					
Alpha-Tocopherol (AT)	33.8	19.6	30.3	23.9	19.5
Beta-Carotene (BC)	21.1	30.1	23.3	24.6	25.4
AT and BC	21.8	23.3	23.2	23.9	29.7
Placebo	23.3	27.1	23.2	27.7	25.4

[a]Based on total number of controls $n = 656$.
[b]Units of measurement = number of copies of mtDNA per cell.
[c]Mean values unless otherwise noted.
[d]Adjusted by total energy intake. Number of controls = 609.
[e]Number of controls = 642; Postintervention serum measures limited to subjects in the intervention arm only.

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Table 3. Crude and adjusted ORs and 95% CIs for mtDNA copy number and pancreatic cancer risk

	mtDNA copy number						
	Q1 ≤94.6	Q2 >94.6–≤113.3	Q3 >113.3–≤132.8	Q4 >132.8–≤158.0	Q5 >158.0	P_{trend}[a]	Continuous OR[b]
n (cases/controls)	38/133	39/133	30/142	43/130	53/118		203/656
Crude	1.00 (ref)	1.03 (0.62–1.71)	0.74 (0.43–1.26)	1.16 (0.70–1.91)	1.57 (0.97–2.55)	0.06	1.13 (1.05–1.22)
Adjusted[c]	1.00 (ref)	1.04 (0.63–1.74)	0.75 (0.44–1.28)	1.22 (0.74–2.02)	1.64 (1.01–2.67)	0.04	1.14 (1.06–1.23)
Cases diagnosed <7 years after whole blood collection							
n (cases/controls)	38/118	23/130	13/142	21/133	23/133		1,18/656
Crude	1.00 (ref)	0.91 (0.48–1.73)	0.53 (0.26–1.10)	1.02 (0.55–1.91)	1.86 (1.05–3.31)	0.05	1.13 (1.05–1.22)
Adjusted[c]	1.00 (ref)	0.78 (0.38–1.59)	0.60 (0.28–1.27)	1.16 (0.59–2.27)	2.14 (1.16–3.96)	0.01	1.21 (1.10–1.34)
Cases diagnosed ≥7 years after whole blood collection	15/118	20/130	17/142	18/133	15/133		85/656
Crude	1.00 (ref)	1.20 (0.58–2.48)	1.06 (0.51–2.21)	1.36 (0.67–2.78)	1.13 (0.53–2.40)	0.06	1.03 (0.92–1.16)
Adjusted[c]	1.00 (ref)	1.27 (0.61–2.63)	1.08 (0.52–2.27)	1.37 (0.67–2.81)	1.14 (0.53–2.45)	0.67	1.05 (0.93–1.18)

[a]Test for trend calculated using the median value for each mtDNA copy number quintile.
[b]Continuous variables are presented based on a 21 unit change, which corresponds to the average unit change within quintiles 2–4.
[c]Adjusted for age, smoking duration, smoking intensity, diabetes.

quintile of mtDNA copy number. No clear, consistent trend existed for the selected baseline characteristics and mtDNA copy number. Men in the lowest quintile of mtDNA copy number tended to have smoked the most cigarettes for a greater number of years and had the highest number of pack-years ($P_{trend} = 0.05$).

mtDNA copy number was significantly associated with pancreatic cancer risk (highest mtDNA copy number quintile compared with lowest quintile, OR = 1.64, 95% CI = 1.01–2.67, $P_{trend} = 0.04$) and in continuous models (OR = 1.14, 95% CI = 1.06–1.23; Table 3). We also conducted a dichotomous analysis by splitting mtDNA copy number at the median (OR = 1.25, 95% CI = 1.01–1.79). Similar associations were observed when comparing quartiles of mtDNA copy number (adjusted OR for 4th quartile vs. 1st quartile = 1.21, 95% CI = 1.01–1.34; test for trend $P = 0.09$) and when we restricted our analysis to the matched case–control sets and used conditional logistic regression to estimate risk (highest compared with lowest quintile, OR = 1.23, 95% CI = 0.62–2.43, continuous OR = 1.13, 95% CI = 1.02–1.26). Patterns were similar when restricting the analysis to pancreas and lung controls only (adjusted ORs for the 1st–5th quantiles in order = 1.00, 0.76, 0.54, 0.94, 1.15; continuous OR = 1.10, 95% CI = 1.01–1.19) and to pancreas and lymphoma controls only (adjusted ORs for the 1st–5th quantile in order = 1.00, 0.82, 0.63, 1.08, 1.43; continuous OR = 1.12, 95% CI = 1.04–1.22). The association between mtDNA copy number and pancreatic cancer was strongest in cases diagnosed during the first 7 years of follow-up (highest mtDNA copy number quintile OR = 2.14, 95% CI = 1.16–3.96, $P_{trend} = 0.01$, continuous OR = 1.21, 95% CI = 1.10–1.34, $P = 0.02$), but not in cases occurring during follow-up greater than 7 years (highest mtDNA copy number quintile OR = 1.14, 95% CI = 0.53–2.45, continuous OR = 1.05, 95% CI = 0.93–1.18).

Study intervention group did not significantly modify the association between mtDNA copy number and pancreatic cancer risk, although in adjusted models, positive associations seemed strongest among those randomized to the alpha-tocopherol supplementation group (continuous OR = 1.30, 95% CI = 1.09–1.57, $P_{trend} = 0.004$; data not shown). However, there was no significant interaction by baseline serum alpha-tocopherol and serum alpha-tocopherol measured after the study intervention. The association between mtDNA copy number and pancreatic cancer was not modified by age, smoking habits, BMI, physical activity, saturated fat, total energy, folate or carbohydrate intake, other dietary factors (antioxidants, fruit/vegetable intake), history of diabetes or gallstones, and physical activity levels (all $P > 0.05$).

Discussion

To our knowledge, this is the first study to evaluate and show a statistically significant positive association between higher mtDNA copy number and pancreatic cancer risk.

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The association was more pronounced among cases that occurred during the first 7 years of follow-up. In our study, the mean mtDNA copy number for controls was 126.9, and the log-transformed mean was around 4.8. Antioxidants and other dietary factors believed to lower mtDNA copy number did not seem to affect copy number in this analysis. Our mtDNA copy number measures may be higher than previously reported studies because our participants are current smokers and smoking increases oxidative stress, which is known to increase mtDNA copy number (7).

Most studies investigating the association between mtDNA copy number and cancer are case–control (28–32) or nested case–control (23, 24) designs, comparing cancer cases to normal controls or normal tissue to tumors at different stages. Inconsistent associations have been observed between mtDNA copy number measured in peripheral blood or tumor tissue for certain cancers, including renal cell carcinoma (28, 33), non-Hodgkin lymphoma (23), breast (29, 34), head and neck (35, 36), endometrium (30), stomach (31) lung (32,24), and ovarian (37) cancer. Specifically, both positive and inverse associations have been observed across cancer sites and, in some instances, within cancer sites (i.e., ovary, head and neck cancers), regardless whether mtDNA copy number was measured in blood or tissue. These inconsistencies may reflect true differences in mtDNA levels within or across the tumor types. Alternatively, the inconsistencies might be due to differences in study designs (i.e., case control vs. prospective nested case control) or laboratory methods used to quantify mtDNA (11). For example, mtDNA copy number was higher in early-stage ovarian tumors, whereas late-stage tumors had lower mtDNA copy number (37). A recent biological study on gastric cancer tissue suggested that mtDNA instability may play a role in early events leading to cancer progression (37). These findings suggest that changes in mtDNA content might be a genetic event in the progression of carcinogenesis (37, 38). Because we are using a nested case–control study approach, we were able to assess the possibility of reverse causation and show that mtDNA copy number was elevated prior to diagnosis, unlike case–control studies that cannot ascertain temporal exposure in relation to disease. As the associations we observe are more pronounced among cases occurring earlier during follow-up, our results suggest increased levels of mtDNA copy number may be an early biomarker of subclinical pancreatic cancer in certain populations.

The strength of our study is its prospective nature, with blood samples and other risk factor data collected prior to cancer diagnosis. The cases and controls were both obtained from the larger cohort study; therefore, our study has internal validity, and no survival bias of cases or selection bias of controls. Our findings in male smokers, however, may not be generalizable to populations that include women and nonsmokers. In particular, the association between mtDNA copy number and pancreatic cancer may be different in populations not exposed to factors that induce oxidative stress, such as cigarette smoke (12, 13). Residual confounding by cigarette smoking is possible but unlikely because all subjects were current smokers at baseline, self-reported current smoking is highly accurate in adults (39), and the smoking exposures were not effect modifiers in any association. In addition, the association was the same when we limited the analysis to those who smoked 20 cigarettes per day, which is equivalent to 1 pack.

Our study also has limitations. We did not have repeated measures of mtDNA copy number and a single measurement may not reflect mtDNA copy number over a lifetime. Repeated measures may help clarify the temporal relationship between mtDNA copy number and pancreatic cancer development. In addition, the number of cases in our study is relatively small, particularly to evaluate interactions.

In conclusion, we found a significant association between higher mtDNA copy number and increased pancreatic cancer risk, particularly for cases diagnosed within 7 years of whole blood collection. Further research is needed to evaluate our findings in other populations and to explore the potential use of mtDNA copy number as an early detection biomarker.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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