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Cancer Epidemiology, Biomarkers & Prevention

Null Results in Brief

Pooled Analysis of Mitochondrial DNA Copy Number and Lung Cancer Risk in Three Prospective Studies

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Abstract

Background: We previously reported that higher levels of mitochondrial DNA copy number (mtDNA CN) were associated with lung cancer risk among male heavy smokers (i.e., ≥20 cigarettes per day) in the Alpha-Tocopherol Beta-Carotene (ATBC) study. Here, we present two additional prospective investigations nested in the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial and the Shanghai Women's Health Study (SWHS), and pooled with previously published data from ATBC.

Materials: All DNA were extracted from peripheral whole blood samples using the phenol–chloroform method, and mtDNA CN was assayed by fluorescence-based qPCR. Multivariate unconditional logistic regression models were used to estimate ORs and 95% confidence intervals for the association of mtDNA CN and lung cancer risk.

Results: Overall, mtDNA CN was not associated with lung cancer risk in the PLCO, SWHS, or pooled populations (all P trends > 0.42, P heterogeneity = 0.0001), and mtDNA CN was inversely associated with lung cancer risk among male smokers in PLCO, the opposite direction observed in ATBC. In addition, the mtDNA CN association observed among male heavy smokers in ATBC was the opposite direction in PLCO.

Conclusions: mtDNA CN was not consistently associated with lung cancer risk across three prospective study populations from Europe, Asia, and the United States.

Impact: This pooled study suggests no consistent association between prediagnostic mtDNA CN levels and lung cancer risk across several populations. *Cancer Epidemiol Biomarkers Prev; 23(12); 2977–80. ©2014 AACR.*

Introduction

Mitochondria are primarily responsible for energy production in eukaryotic cells (1). Mitochondria have a singular circular mitochondrial DNA molecule. Mitochondria copy number (mtDNA CN) varies to meet energy needs and cope with oxidative stress (2). Oxidative stress disrupts homeostasis and forms reactive oxygen species leading to DNA damage and genomic instability (3). In a prospective study among Finnish male smokers, we observed a positive association between mtDNA CN in peripheral blood leukocytes and lung cancer risk (4).

To replicate the mtDNA CN–lung cancer risk association, nested case–control studies were conducted in two prospective studies, the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial, a population of men and women across the United States, and the Shanghai Women's Health Study (SWHS), a population of mostly nonsmoking Chinese women. These data were then pooled with published data from the Alpha-Tocopherol Beta-Carotene (ATBC) study (4).

Materials and Methods

Study subjects

The ATBC (5), PLCO (6), and SWHS (7) studies have been described in detail. From ATBC, a total of 229 incident cases with an available blood specimen for analysis were previously identified (4). Controls were matched to cases 1:1 based on date of birth. From PLCO, a total of 442 cases of first primary incident lung cancer with serum at baseline from the screening arm were identified. Controls were matched by age, sex, race, blood collection date, and diagnosing center. From SWHS, 226 incident lung cancer

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Table 1. Demographic and mtDNA CN characteristics of ATBC, PLCO, and SWHS populations

		Cases		Controls		
Study (Ca/Co)	**Characteristic**	**Mean/*N***	**SD/%**	**Mean/*N***	**SD/%**	***P* value[a]**
ATBC	BMI (kg/m^2)	25.59	3.49	26.35	3.87	0.045
(227/227)	Age	56.67	5.00	58.41	4.79	0.68
	Years smoking	38.47	7.11	35.83	9.22	0.005
	Ever smoker	227	100	227	100	1
	mtDNA CN	134.23	45.27	127.85	36.07	0.26
	median, IQR	125.86	(102.04–160.18)	124.79	(100.63–151.51)	
PLCO	BMI (kg/m^2)	26.84	4.39	27.43	4.36	0.028
(426/436)	Age	64.07	4.95	63.67	4.71	0.22
	Years smoking	34.89	15.15	15.57	17.47	<0.0001
	Ever smoker	380	89.20	238	54.59	<0.0001
	mtDNA CN	128.37	128.26	127.39	59.96	0.079
	median, IQR	110	(87–140)	114	(90–145)	
	Sex					
	Male	259	60.80	267	61.24	0.89
	Female	167	39.20	169	38.76	
	Race					
	White	399	93.66	404	92.66	0.81
	Black	15	3.52	18	4.13	
	Other	12	2.82	14	3.21	
	Center					
	University of Colorado	23	5.40	23	5.28	1
	Georgetown University	19	4.46	19	4.36	
	Pacific Health Research and Education Institute	9	2.11	9	2.06	
	Henry Ford Health System	66	15.49	63	14.45	
	University of Minnesota	126	29.58	131	30.05	
	Washington University	50	11.74	50	11.47	
	University of Pittsburgh	43	10.09	46	10.55	
	University of Utah	29	6.81	31	7.11	
	Marshfield Clinic Research Foundation	45	10.56	47	10.78	
	University of Alabama Birmingham	16	3.76	17	3.90	
SWHS	BMI (kg/m^2)	24.58	3.45	25.01	3.53	0.16
(221/222)	Age	59.22	8.20	59.18	8.35	0.93
	Years smoking	2.97	10.67	1.74	8.19	0.23
	Ever smoker	17	7.69	11	4.95	0.24
	mtDNA CN	191.84	315.45	185.88	268.6	0.82
	median, IQR	118	(94–158)	124	(93–162)	

Abbreviations: Ca, cases; Co, controls; IQR, interquartile range.

[a]Wilcoxon rank-sum test for continuous variables/Pearson χ^2 for categorical variables.

cases with adequate blood for mtDNA analysis were identified, with one control matched to case by age, smoking status, and date of blood collection.

Laboratory analysis

In all three studies, DNA was extracted from peripheral whole blood by the phenol–chloroform method. mtDNA CN was assayed in the same laboratory by qPCR using the ratio of the estimated threshold cycle number of *ND1* mitochondrial gene and the B-globin nuclear gene, *HBB* (8). The coefficient of variation for PLCO, SWHS, and ATBC was 14%, 7%, and 13%, respectively. After excluding bad runs, poor-quality DNA, or subjects with missing demographic variables, 227 cases and 227 controls in ATBC, 426 cases and 436 controls in PLCO, and 221 cases and 222 controls in SWHS were included in the final analysis.

Statistical analysis

Differences between cases and controls for demographic characteristics were tested with the Wilcoxon-signed rank-sum test for continuous variables and the Pearson χ^2

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Table 2. mtDNA copy number and risk of lung cancer across ATBC, PLCO, and SWHS studies by sex and smoking status

	ATBC (Ca 227/Co 227)			PLCO (Ca 426/Co 436)			SWHS (Ca 221/Co 222)			Pooled		
mtDNA%	Ca/Co	OR[a]	95% CI	Ca/Co	OR[a]	95% CI	Ca/Co	OR[a]	95% CI	Ca/Co	OR[a]	95% CI
Overall												
Q1	52/57	1.00	(ref.)	117/107	1	(ref.)	54/56	1.00	(ref.)	223/220	1.00	(ref.)
Q2	59/56	1.34	(0.71–2.37)	119/110	0.98	(0.64–1.50)	72/58	1.27	(0.48–1.47)	250/224	1.08	(0.82–1.42)
Q3	43/57	1.15	(0.61–2.17)	96/108	0.84	(0.55–1.29)	44/53	0.84	(0.48–1.47)	183/218	0.83	(0.62–1.11)
Q4	73/57	2.38	(1.12–5.06)	94/111	0.98	(0.63–1.52)	51/55	0.91	(0.53–1.57)	218/223	1.04	(0.79–1.39)
	P trend		0.049	*P* trend		0.74	*P* trend		0.42	*P* trend		0.78
Female												
Q1		—	—	32/40	1	(ref.)	54/56	1.00	(ref.)	86/96	1.00	(ref.)
Q2		—	—	38/41	1.51	(0.74–3.11)	72/58	1.27	(0.48–1.47)	110/99	1.34	(0.89–2.02)
Q3		—	—	41/44	1.47	(0.73–2.98)	44/53	0.84	(0.48–1.47)	85/97	1.03	(0.67–1.58)
Q4		—	—	56/44	2.12	(1.07–4.22)	51/55	0.91	(0.53–1.57)	107/99	1.24	(0.82–1.89)
				P trend		0.043	*P* trend		0.42	*P* trend		0.59
Male												
Q1	52/57	1.00	(ref.)	85/67	1	(ref.)		—	—	137/125	1.00	(ref.)
Q2	59/56	1.34	(0.71–2.37)	81/69	0.76	(0.44–1.28)		—	—	140/125	0.89	(0.61–1.29)
Q3	43/57	1.15	(0.61–2.17)	55/64	0.59	(0.34–1.05)		—	—	98/121	0.69	(0.46–1.02)
Q4	73/57	2.38	(1.12–5.06)	38/67	0.55	(0.29–0.99)		—	—	111/124	0.92	(0.62–1.35)
	P trend		0.049	*P* trend		0.028				*P* trend		0.39
Never smoker												
Q1		—	—	9/51	1	(ref.)	52/53	1.00	(ref.)	61/104	1.00	(ref.)
Q2		—	—	11/51	1.35	(0.49–3.72)	66/58	1.15	(0.68–1.94)	77/109	1.17	(0.74–1.85)
Q3		—	—	12/42	1.23	(0.43–3.57)	40/51	0.79	(0.44–1.40)	51/93	0.86	(0.52–1.41)
Q4		—	—	14/54	1.49	(0.55–4.08)	46/49	0.94	(0.54–1.65)	60/103	1.03	(0.64–1.68)
				P trend		0.49	*P* trend		0.54	*P* trend		0.79
Ever smoker												
Q1	52/57	1.00	(ref.)	108/56	1	(ref.)	2/3	1.00	(ref.)	162/116	1.00	(ref.)
Q2	59/56	1.34	(0.71–2.37)	108/59	0.91	(0.56–1.47)	6/0	—	—	173/115	1.03	(0.73–1.47)
Q3	43/57	1.15	(0.61–2.17)	84/66	0.72	(0.44–1.17)	4/2	4.33	(0.24–79.49)	132/125	0.78	(0.54–1.12)
Q4	73/57	2.38	(1.12–5.06)	80/57	0.84	(0.50–1.39)	5/6	1.05	(0.087–12.81)	158/120	1.05	(0.73–1.49)
	P trend		0.049	*P* trend		0.33	*P* trend		0.46	*P* trend		0.81
Male smoker												
Q1	52/57	1.00	(ref.)	78/38	1	(ref.)		—	—	130/95	1	(ref.)
Q2	59/56	1.34	(0.71–2.37)	78/40	0.8	(0.45–1.45)		—	—	137/96	0.94	(0.63–1.39)
Q3	43/57	1.15	(0.61–2.17)	53/45	0.58	(0.32–1.06)		—	—	96/102	0.69	(0.46–1.03)
Q4	73/57	2.38	(1.12–5.06)	33/37	0.50	(0.25–0.98)		—	—	106/94	0.95	(0.63–1.43)
	P trend		0.049	*P* trend		0.021				*P* trend		0.45

NOTE: %Quartiles: ATBC: <100.6, ≥100.6–124.8, ≥124.8–151.5, ≥151.5; PLCO: <90, ≥90–114, ≥114–145, ≥145; SWHS: <93, ≥93–124, ≥124–162, ≥162; Median: ATBC: ≤124.8; PLCO: ≤114, SWHS: <124.

Abbreviations: Ca, cases; Co, controls; Q1–Q4, quartiles.

[a]Adjusted for age, BMI, pack-years, race, sex, date of enrollment, center (if applicable), and study.

test for categorical variables. mtDNA CN was categorized by quartiles among controls in each study. Unconditional logistic regression models generated ORs and 95% confidence intervals (95% CI) to estimate the association of mtDNA CN and risk of lung cancer. Models were adjusted for age, body mass index (BMI), race, pack-years smoking, and date of blood collection. PLCO was additionally adjusted for sex and study center. Pooled analyses were additionally adjusted for study. Additional analyses were stratified by sex, smoking status, and smoking pack-years. P trend modeled mtDNA CN quartiles continuously. Between-study heterogeneity was tested by random effects with the rmeta package [Thomas Lumley (2012). rmeta: Meta-analysis. R package version 2.16.

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http://CRAN.R-project.org/package=rmeta]. Analyses were performed in SAS 9.3 (SAS Institute).

Results

Selected demographic characteristics of the study population are described in Table 1. Statistically significant differences between cases and controls in BMI and years of smoking were observed in the ATBC and PLCO populations but not in SWHS. Age, sex, race (all Caucasian in ATBC, Asian in SWHS), and mtDNA CN levels were not different between cases and controls across the three populations.

mtDNA CN was not associated with lung cancer risk in the PLCO or SWHS data; pooled analysis was also null with evidence of heterogeneity across studies (P heterogeneity = 0.0001; Table 2). mtDNA CN and lung cancer risk associations were inverse in male smokers in PLCO, the opposite direction of ATBC. Similarly, comparing heavy smokers to non/light smokers, associations in PLCO were the opposite direction of ATBC (data not shown). No differential associations were observed by histology or follow-up time (data not shown).

Discussion

The mtDNA CN and lung cancer risk association observed in ATBC did not replicate in PLCO, SWHS, and pooled study populations. There was no consistent evidence of an association across populations by sex or smoking status/intensity. This pooled study suggests no consistent association between prediagnostic mtDNA CN levels and lung cancer risk across several populations.

Our study included diverse study populations. Additional strengths included the combined large sample size and the standardized specimen processing and mtDNA CN assay across the three studies. The primary weakness of this study was the single measurement of mtDNA CN. Copy number could change over time, and this study was unable to determine intrapersonal variation over time.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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