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Mitochondrial DNA copy number and chronic lymphocytic leukemia/small lymphocytic lymphoma risk in two prospective studies

Christopher Kim[1], **Bryan A. Bassig**[1], **Wei Jie Seow**[1], **Wei Hu**[1], **Mark P. Purdue**[1], **Wen-Yi Huang**[1], **Chin-San Liu**[2], **Wen-Ling Cheng**[2], **Satu Männistö**[3], **Roel Vermeulen**[4], **Stephanie J. Weinstein**[1], **Unhee Lim**[5], **H. Dean Hosgood**[6], **Matthew R. Bonner**[7], **Neil E. Caporaso**[1], **Demetrius Albanes**[1], **Qing Lan**[1,*], and **Nathaniel Rothman**[1,*]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA [2]Neurology and Vascular and Genomic Center, Changhua Christian Hospital, Changhua, Taiwan [3]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland [4]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands [5]Epidemiology Program, University of Hawaii Cancer Center, Honolulu, Hawaii, USA [6]Albert Einstein College of Medicine, Yeshiva University, Bronx, NY, USA [7]Department of Epidemiology and Environmental Health, State University of New York at Buffalo

Abstract

Background—Mitochondrial DNA copy number (mtDNA CN) may be modified by mitochondria in response to oxidative stress. Previously, mtDNA CN was associated with non-Hodgkin lymphoma (NHL) risk, particularly chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). We conducted a replication study in the Prostate, Lung, Colorectal, and Ovarian (PLCO) study and pooled with published ATBC (Alpha-Tocopherol, Beta-Carotene) data.

Methods—In PLCO, 292 NHL cases (95 CLL/SLL cases) and 301 controls were pooled with 142 NHL cases (47 CLL/SLL cases) and 142 controls from ATBC. Subjects answered a questionnaire and provided blood. DNA was extracted from pre-diagnostic peripheral white blood, and mtDNA CN assayed by quantitative polymerase chain reaction. Unconditional logistic regression estimated mtDNA CN and NHL risk by odds ratios (OR) and 95% confidence intervals (95% CI).

Results—Greater mtDNA CN was associated with increased risk of CLL/SLL among males in PLCO (3rd vs. 1st tertile: OR: 2.21; 95% CI: 1.03–4.72; p-trend: 0.049) and pooled (T3 vs. T1 OR: 3.12; 95% CI: 1.72–5.68; p-trend: 0.0002). Association was stronger among male smokers (p-trend: <0.0001) and essentially identical for cases diagnosed <6, >6–8, and >8 years from blood

Corresponding author: Christopher Kim, 9609 Medical Center Drive, MSSC 9771, Bethesda, MD, 20892, 240-276-7440, christopher.kim@nih.gov.
*These authors co-supervised this work

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draw (pooled: p-interaction: 0.65). mtDNA CN and risk of other NHL subtypes and multiple myeloma showed no association.

Conclusions and Impact—Mitochondrial DNA CN was associated with risk of CLL/SLL in males/male smokers. The risk was observed among cases diagnosed as long as eight years after blood draw. These results suggest that higher mtDNA CN may reflect a process involved in CLL/SLL development.

Keywords

Mitochondrial DNA copy number; chronic lymphocytic leukemia/small lymphocytic lymphoma; non-Hodgkin lymphoma

Introduction

Mitochondria are the principal organelle in eukaryotic cells responsible for energy production through the generation of adenosine triphosphate (ATP) via the electron transport chain (ETC)(1). Mitochondria have a singular circular mitochondrial DNA (mtDNA) molecule that is approximately 16k-bp long. As mitochondria age, generation of reactive oxygen species (ROS) increases. This intracellular production of ROS increases oxidative stress, causing damage to DNA, inducing modification of the purine and pyrimidine bases, single and double-strand breaks, and cross-links to other molecules (2). As mitochondria experience more oxidative stress, pro-inflammatory cytokine production increases (3), which has been associated with risk of non-Hodgkin lymphoma (NHL) (4).

Mitochondrial DNA lacks the protective histones and repair capacity of nuclear chromosomal DNA(2). Expression and stability of mtDNA has been suggested to play a critical role in human pathogenesis due to deficiency in maintenance and stability of the ETC(5), and mitochondria may modify genomic copy number as a mechanism to cope with increased genomic instability and damage (6). Measurements in blood samples of subjects have shown that oxidative stress measurements by thiobarbituric acid reactive substances, 8-hydroxy-2′-deoxyguanosine, and 4,977bp deletions and mtDNA CN have been positively correlated (7).

Several studies have examined variation in mtDNA copy number (mtDNA CN) and cancer risk; most prospective studies have found apositive association and retrospective studies have found an inverse association (8–17), including a strong association observed between increased mtDNA copy number and risk of NHL, particularly CLL/SLL, in a prospective cohort study of Finnish male smokers (13) and Europeans (18). Liao et al. noted an increased risk in gastic cancer cases diagnosed recently in relation to blood draw/entry into study (≤2 years) in a cohort of Chinese women (19), suggesting that mtDNA CN could be influenced by the result of disease and more prospective data are needed. Contrastingly, low mtDNA CN has been associated with lung tumor (20), breast cancer (21), and ovarian cancer (22) progression.

To confirm previous findings of mtDNA CN and risk of NHL/CLL/SLL, we conducted a nested case-control study in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer

Screening Trial and pooled previously published data (13) from the Alpha-Tocopherol, Beta-Carotene cancer prevention study (ATBC). Additionally, we utilized the newest case definitions of NHL subtypes from the Pathology Working Group of the International Lymphoma (INTERLYMPH) Epidemiology Consortium (23) to update the previously published ATBC study.

Materials and Methods

Study subjects

The PLCO screening trial has been described in detail (24). Briefly, PLCO is a large randomized trial to determine if screening for these cancers reduces cause-specific mortality. A total of 154,910 males and females aged 55–74 with no prior history of cancer were enrolled during 1993–2001 in 10 different centers around the United States. Self-administered questionnaire inquired information on demographics and risk factors as well blood specimens for intervention-arm participants. Within two hours of blood collection, specimens were processed and frozen at −70°C. The PLCO study was reviewed by the institutional review boards of the National Cancer Institute and the 10 study centers and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Cancer cases in the PLCO trial were identified through annual follow-up questionnaires and telephone calls. First primary cancers of the P, L, C and O were also identified through screening tests conducted in the first six years of the trial. We identified301 cases of first primary lymphoid malignancies (244 NHL cases; 95 CLL/SLL cases; 57 multiple myeloma cases) with serum collected at baseline from the screening arm in PLCO. These cases were confirmed by medical record review at each screening center. Controls were individually matched to cases (1:1 ratio) by age at baseline (5-year categories), sex, race, and interview/blood collection date (±3 months) from among subjects who had not been diagnosed with any type of malignancy except non-melanoma skin cancer at the time of the case diagnosis date.

The ATBC study has been described in detail (25). Briefly, 29,133 eligible male smokers, aged 50 to 69 years, were recruited from southwest Finland and randomized in 1985–1988 to determine whether supplementation with α-tocopherol or β-carotene reduced the incidence of lung and other cancers. At the first visit, the males completed interviews with a study nurse on basic demographic characteristics and whole blood was collected at follow-up 1992–1993. All cases of NHL were identified using the Finnish Cancer Registry, which provides virtually 100% of case ascertainment from the ATBC cohort (26). The hospital records of the identified cases were reviewed by an experienced study oncologist for confirmation of the lymphoma diagnosis. Through April 30, 2002, 142 individuals with incident cases of lymphoid neoplasms (122 NHL cases; 47 CLL/SLL cases; 20 multiple myeloma cases) with an available pre-diagnostic blood specimen for analysis were identified. Controls were individually matched to cases (1:1) on date of birth (±5 years). In the previously published data from ATBC(13), 107 cases were utilized; updated INTERLYMPH and SEER classifications of neoplasms account for 15 NHL cases (12 CLL/SLL) that were not previously included in the analyses. The ATBC study was approved by

institutional review boards at the National Cancer Institute and the National Public Health Institute of Finland. Participants provided written informed consent in accordance with the Declaration of Helsinki.

Histologic type was obtained by medical record abstraction or recorded by the tumor registrar at each participating medical center using standard ICD-O-2 codes that were re-coded to ICD-O-3. Subtypes of NHL were categorized by the most recent INTERLYMPH classification update from 2010 (23) utilizing the 5th hierarchical level (ATBC analyses were regrouped to this classification). When anICD-O-3 code was recorded in the PLCO or ATBC records but not defined in INTERLYMPH, codes where identified using NCI's SEER classification for ICD-O-3 coding materials to classify the remaining subtypes (http://seer.cancer.gov/icd-o-3/sitetype.icdo3.d20121205.pdf). The subtype groupings were classified as follows: multiple myeloma (9731, 9732, 9733, 9734), CLL/SLL/B-PLL/MCL (9670, 9673, 9823, 9832, 9833; all cases were 9670 or 9823, referred to CLL/SLL throughout for simplicity), diffuse large B-cell lymphoma (DLBCL) (9678,9679, 9680-9689, 9712, 9735, 9737,9738), follicular (9690-9699) and other/NOS (9590, 9591, 9596, 9673,9675, 9684, 9687, 9704, 9707, 9710, 9715, 9727, 9728, 9765, 9766, 9811-9812, 9820, 9835, 9836, 9940, 9970). Case histology by cohort is presented in Table 1.

Laboratory analysis

DNA was extracted from the peripheral white blood cells of the whole blood using the phenol–chloroform method (27), and fluorescence-based quantitative polymerase chain reaction determined mtDNA CN using the ratio of the estimation of threshold cycle number of ND1, a mitochondrial gene, and the B-globin gene, HBB, a nuclear gene (7). Samples for both studies were processed and assayed by the same laboratory. Cases and controls were blindly assayed consecutively within each batch. Blinded quality control duplicate samples (8 samples from 2 subjects) were interspersed in each batch to evaluate assay reproducibility. In the PLCO analysis, the overall coefficient of variation (CV) of this assay was 6.8%, 9 of 301 selected cases and 2 of 303 selected controls had poor quality DNA or the assay malfunctioned, leaving 292cases and 301 controls in the final analysis. In the ATBC analysis, 142 matched sets of cases and controls were previously assayed, with an overall CV of 13%(13). In total, 434cases and 443 controls comprised the pooled ATBC and PLCO analyses.

Statistical analysis

Differences in selected demographic characteristics between cases and controls were assessed using the Wilcoxon rank sum test for continuous variables and Pearson chi-square test for categorical variables. Unconditional logistic regression models were used to generate odds ratios (OR) and 95% confidence intervals (95% CI) to estimate the association of mtDNA CN and risk of CLL/SLL. Polytomous logistic regression models were used in place of binary logistic regression in analyses stratified by NHL, subtypes (DLBCL, Follicular, Other/NOS), multiple myeloma, and time since blood draw. Cohort-specific distribution of mtDNA CN among all controls was used to determine cutoff points for tertiles. In the pooled analysis, tertile groupings were maintained from each cohort to minimize any potential effects of lab drift. Unconditional model results were similar to

conditional models, but to maximize statistical stability, unconditional models which utilized all controls were used for all analyses. All models compared the higher versus the lowest tertile of mtDNA. Models were adjusted for sex (male, female-PLCO and Pooled only), age (continuous), tobacco (pack-years), BMI (continuous), race (white, black, other-PLCO and Pooled only), date of blood draw (year), and study (PLCO, ATBC). After adjustment for supplementation group in ATBC, results were similar to non-adjusted estimates; thus, supplement group was left out of the final models. We also conducted analyses stratifying by sex (male, female) and smoking status (ever, never). Additionally, we conducted stratified analyses in males by time since blood draw to diagnosis (≤6 years, >6–8 years, >8 years). P-trend utilized the tertiles of mtDNA modeled continuously. Significance tests were two-sided at the 0.05 level. All analyses were conducted in SAS 9.22 (SAS Institute).

Results

The demographic characteristics were fairly similar between the lymphoid cases, CLL/SLL cases, and controls for both studies (Table 1). Sex, tobacco, race, age, BMI, and pack-years of tobacco smoked were not statistically different between CLL/SLL cases and controls (P-value > 0.05). mtDNA copy number was significantly higher in cases than controls in the ATBC study (P-value < 0.05). Being male and pack-years of smoking were negatively associated with mtDNA CN among controls (pooled: linear regression, B: −37.97, −0.26 respectively, P-value < 0.05). The range of mtDNA CN was wider in PLCO than in ATBC and the median mtDNA CN was greater in PLCO (median of controls: 121) than ATBC (median of controls: 109). There was no significant difference (p-value: 0.11) in mtDNA CN comparing male smoker controls in PLCO (mean: 128.3) versus controls in ATBC (mean: 117.7) (data not shown).

Mitochondrial DNA copy number was not associated with risk of NHL in PLCO (Supplementary Table 1). Mitochondrial DNA copy number was elevated but not significantly associated with risk of CLL/SLL in PLCO (highest vs. lowest tertile, OR: 1.53; 95% CI: 0.84–2.81), but was significantly associated in the pooled population (OR: 2.25; 95% CI: 1.35–3.74) (Table 2). In males, the elevated risk of CLL/SLL was observed in both studies individually and the pooled analysis (P-trend <0.05, P-interaction by sex < 0.05), especially among smokers (highest vs. lowest tertile: OR: 4.28; 95% CI: 2.06–8.88). When PLCO analyses were restricted to the characteristics of the ATBC population (males and ever smokers), CLL/SLL risk was elevated (OR: 2.93; 95% CI: 1.01–8.51). Compared to the PLCO population, the effect estimates in the ATBC population were greater although not statistically different (p-value: 0.13). There was suggestion of no association in non-smokers, and there was no association in females. Additional stratified analyses by time to diagnosis from blood draw among males showed a consistent elevation of risk at ≤6, 6–8, and >8 years in the pooled population (P-interaction: 0.65, Table 3). Analyses with non-whites removed were not substantially different, and stratified analyses by age and BMI were not dramatically different or statistically unstable (data not shown).

When the analyses were conducted for NHL, NHL subtypes, or multiple myeloma, no significant associations were observed in the pooled data (p-trend > 0.05) (Supplemental

Table 1). Analyses for overall NHL and mtDNA CN associations stratified by sex, smoking status, and time from blood draw to diagnosis were unremarkable (Supplementary Table 2 and 3).

Discussion

We pooled data from two prospective studies, that consisted of new data from PLCO and previously published data from ATBC(13). We found consistent evidence of an association between increased mtDNA CN and CLL/SLL risk among males and male smokers in the PLCO study and the pooled study. By pooling data, we were able to observe the mtDNA-CLL/SLL risk association in cases diagnosed as long as 8+ years after baseline blood draw. Similar to data previously published (13, 18), these results suggest mtDNA CN could reflect a process that reflects CLL/SLL development. There was no evidence of an association between mtDNA CN and CLL/SLL in non-smokers; similarly, there was no observed association in females, although limited by few cases. We observed no association with mtDNA CN and risk of overall NHL, other NHL subtypes, or multiple myeloma risk.

In general, studies of mtDNA CN and risk of cancer have observed a positive association at various sites in prospective studies: lung (9, 16), cervix (11), colorectal (14, 17), and pancreas (15). One prospective study did not observe an overall association with gastric cancer risk in females but cases diagnosed within two years of blood draw had an increased risk, suggesting mtDNA CN elevation was an effect of the disease (19). Consistent with other prospective studies, but unlike the aforementioned gastic cancer study (19), this study observed an mtDNA CN association with CLL/SLL risk in male smokers, similar to a previously published study (13). However, the strength of the association was slightly weaker in the new PLCO data and more similar to a study in Europeans (18). As leukocytes are from the same target tissue as CLL/SLL, mtDNA measured leukocytes could be a direct indication of mitochondrial activity and regulation which could be a reflection of a disease process that results from genomic damage, oxidative stress, and inflammation occurring in the lymphatic system. Studies where mtDNA was measured in subjects with disease, inverse associations were observed in oral cancer (8), soft-tissue sarcoma (10), and renal cell carcinoma (12) and tumor progression (20–22), suggesting mtDNA CN levels decrease as cancer progresses. One study of colorectal cancer was retrospective, but found a positive association with mtDNA CN (13).

While stratified analyses in other reports have been limited, several studies of mtDNA CN and cancer risk have observed significant associations among smokers and males but not in females. Two studies found the association was strongest among smokers for oral cancer and pancreatic cancer (8, 15). Two studies conducted in male-only populations observed significant associations with lung cancer and pancreatic cancer (9, 15). Three studies did not find significant associations among females for gastric cancer (entire study population was female)(19), renal cell carcinoma (~40% of study was female)(12), and colorectal cancer (~40% cases, ~60% controls of study was female)(17). However, due to relatively few females in our study, the analyses by sex were somewhat underpowered. Thus, we cannot state definitively there was no association in females--only that there was limited suggestion of an association.

Male smokers had increased risk of CLL/SLL when mtDNA CN was elevated. Tobacco smoking (PLCO, ever: 133, never: 155) and being male (PLCO, males: 115, females: 137) were negatively associated with mtDNA CN in controls in this study. Similar trends by sex and smoking status were also observed in a population of Koreans (28). Since male smokers had a lower baseline mtDNA CN, and male smokers with higher mtDNA CN were at increased risk of CLL/SLL, these individuals may have genomic characteristics that are ideal for CLL/SLL development. Mitochondrial DNA genomes from CLL/SLL patients contain high levels of mtDNA content (29), are very stable, and can create genomic copies with deletions and mutations (30) without undergoing apoptosis or senescence.

In the time-based analyses, the mtDNA CN association was similar across several time points up to eight years after blood draw until diagnosis, which suggests that mtDNA CN could reflect a risk factor for CLL/SLL development. Additionally, approximately 20% (n=27 of 142) of cases were in the first tertile, a disproportionately small proportion of cases. While these analyses suggest mtDNA CN could be an etiologic biomarker in CLL/SLL risk, the number of cases available for these stratified analyses were somewhat sparse, so these results should be interpreted with caution.

Individuals with a symptomatic CLL/SLL, mtDNA CN could be an indicator of disease development. Pre-cursor condition to CLL/SLL, monoclonal B-cell lymphocytosis, is detectable in CLL/SLL patients up to 77 months prior to diagnosis (31). But in healthy individuals, mtDNA CN and lymphocytes were not correlated (r: 0.029, p-value: 0.72, n=140); suggesting mtDNA CN is not just a surrogate marker of lymphocytosis.

The primary strengths of this study were due to the prospective designs of the PLCO and ATBC studies. All subjects in this study were recruited prior to diagnosis of any cancer, permitting analyses of associations stratified by the time of blood collection in relation to diagnosis. We assessed mtDNA in white blood cells, the target tissue for CLL/SLL. We utilized the latest revision of the INTERLYMPH working group definitions for histologic subtype classifications of NHL(23) which allowed for more specific analyses of lymphoma subtypes. These revised classifications allowed for more accurate analyses of what are heterogeneous diseases. 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. Exactly how mtDNA changes as disease development progresses is currently unclear. Additionally, there may be residual confounding that may alter mtDNA CN. There was limited evidence of an association in women and non-smokers in PLCO, but only 39% (n=230) of the study subjects were female and 52% (n=303) were non-smokers. Smaller sample size of females and non-smokers compared to males and smokers (particularly when combined with ATBC study subjects) restricted the statistical stability of stratified analyses.

In conclusion, we observed that mtDNA CN was associated with risk of developing CLL/SLL in males and male smokers in PLCO which replicated the results previously found in ATBC. After pooling PLCO and ATBC data, the association was observable as long as eight years after entry into the study suggesting that mtDNA CN may reflect a process that contributes to CLL/SLL development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics of lymphoid cases and controls in PLCO and ATBC nested case-control studies

	PLCO							ATBC						
	Cases (n=292)		CLL/SLL (n=95)		Controls (n=301)			Cases (n=142)		CLL/SLL (n=47)		Controls (n=142)		
Characteristic	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**P-value***	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**P-value***
Sex														
Male	178	(62%)	57	(60%)	185	(61%)	0.85	142	(100%)	47	(100%)	142	(100%)	-
Female	114	(38%)	38	(40%)	116	(39%)		0	-	0	-	0	-	
Tobacco Use														
Current	116	(40%)	36	(38%)	130	(43%)	0.64	123	(87%)	36	(77%)	105	(74%)	0.56
Former	19	(6%)	10	(10%)	25	(8%)		19	(13%)	11	(23%)	37	(26%)	
Never	157	(54%)	49	(52%)	146	(49%)		0	-	0	-	0	-	
Male Ever Smoker														
Yes	90	(31%)	30	(32%)	109	(36%)	0.24	142	(100%)	47	(100%)	142	(100%)	-
No	202	(69%)	65	(68%)	192	(64%)		0	-	0	-	0	-	
Race														
White	278	(94%)	92	(97%)	282	(94%)	0.73	142	(100%)	47	(100%)	142	(100%)	-
Black	5	(2%)	2	(2%)	7	(2%)		0	-	0	-	0	-	
Other	9	(4%)	1	(1%)	12	(4%)		0	-	0		0	-	
NHL Subtype														
DLBCL	48	(16%)						22	(15%)					
Follicular	41	(14%)						12	(8%)					
CLL/SLL	95	(33%)						47	(33%)					
Other/Unknown	55	(19%)						41	(29%)					
Multiple Myeloma	53	(18%)						20	(14%)					
Mean (SD) Age in years	63.97	(5.29)	63.87	(5.34)	63.83	(5.4)	0.77	58.23	(4.81)	59.29	(4.74)	57.64	(4.79)	0.35
Mean (SD) Years of follow-up	5.26	(2.44)	5.04	(2.51)	12.63	(2.15)	<0.0001	8.93	(3.25)	7.7	(2.98)	8.93	(3.25)	0.031
Mean (SD) BMI	27.55	(5.06)	27.01	(4.39)	27.29	(4.03)	0.44	26.36	(4.03)	26.08	(4.33)	26.37	(3.67)	0.66
Mean (SD) Tobacco use (pack-years)	15.59	(25.7)	16.52	(25.71)	19.37	(27.17)	0.41	35.55	(17.24)	35.15	(16.21)	35.83	(17.04)	0.94
Mean (SD) mtDNA	144.3	(97.23)	144.81	(64.34)	144.2	(79.76)	0.33	126.1	(40.84)	137.13	(35.07)	117.7	(38.16)	0.0003
Median (25%–75% range), mtDNA	123	(92–166)	127	(101–176)	121	(89–172)		113	(96–154)	142	(107–161)	109	(93–133)	

* All P-values compare CLL/SLL cases to controls; Chi-squared for categorical variables; Wilcoxon rank sum for continuous variables

Table 2

Association of mtDNA copy number and risk of CLL/SLL in each cohort by sex and smoking status

Cohort		PLCO				ATBC				Pooled			
Characteristic	**mtDNA[$]**	**Ca**	**Co**	**OR[*]**	**95% CI**	**Ca**	**Co**	**OR[#]**	**95% CI**	**Ca**	**Co**	**OR[^]**	**95% CI**
All CLL/SLL	1	22	100	1	(ref)	5	48	1	(ref)	27	148	1	(ref)
	2	38	98	1.77	(0.97–3.23)	14	45	**3.18**	**(1.03–9.83)**	52	**143**	**1.99**	**(1.18–3.36)**
	3	35	103	1.53	(0.84–2.81)	28	49	**5.55**	**(1.89–16.25)**	63	**152**	**2.25**	**(1.35–3.74)**
			P-trend		0.21		P-trend		**0.0017**		P-trend		**0.0022**
Male	1	14	72	1	(ref)	5	48	1	(ref)	19	120	1	(ref)
	2	21	61	1.79	(0.84–3.85)	14	45	**3.18**	**(1.03–9.83)**	35	106	**2.11**	**(1.13–3.92)**
	3	22	52	**2.21**	**(1.03–4.72)**	28	49	**5.55**	**(1.89–16.25)**	50	101	**3.12**	**(1.72–5.68)**
			P-trend		0.049		P-trend		**0.01**		P-trend		**0.0002**
Female	1	8	28	1	(ref)	-	-	-	-	8	28	1	(ref)
	2	17	37	1.66	(0.60–4.56)	-	-	-	-	17	37	1.66	(0.60–4.56)
	3	13	51	0.84	(0.30–2.33)	-	-	-	-	13	51	0.84	(0.30–2.33)
			P-trend		0.59		P-trend				P-trend		0.55
			P-Interaction		**0.019**						P-interaction		**0.0051**
Ever Smoker	1	11	59	1	(ref)	5	48	1	(ref)	16	107	1	(ref)
	2	16	44	2.19	(0.91–5.27)	14	45	**3.18**	**(1.03–9.83)**	30	89	**2.32**	**(1.18–4.55)**
	3	19	52	1.91	(0.82–4.41)	28	49	**5.55**	**(1.89–16.25)**	47	101	**2.94**	**(1.56–5.55)**
			P-trend		0.14		P-trend		**0.01**		P-trend		**0.001**
Never Smoker	1	11	41	1	(ref)	-	-	-	-	11	41	1	(ref)
	2	22	54	1.56	(0.68–3.62)	-	-	-	-	22	54	1.56	(0.68–3.61)
	3	16	51	1.22	(0.49–2.98)	-	-	-	-	16	51	1.22	(0.50–2.98)
			P-trend		0.73		P-trend				P-trend		0.73
			P-Interaction		0.21						P-interaction		0.051
Male Ever Smoker	1	6	45	1	(ref)	5	48	1	(ref)	11	93	1	(ref)
	2	11	31	2.87	(0.96–8.59)	14	45	**3.18**	**(1.03–9.83)**	25	76	**2.94**	**(1.36–6.38)**
	3	13	33	**2.93**	**(1.01–8.51)**	28	49	**5.55**	**(1.89–16.25)**	41	82	**4.28**	**(2.06–8.88)**
			P-trend		0.052		P-trend		**0.01**		P-trend		**<0.0001**
Any Female/Male Non-Smokers	1	16	53	1	(ref)	-	-	-	-	16	53	1	(ref)

Cohort		PLCO				ATBC				Pooled			
Characteristic	**mtDNA[$]**	**Ca**	**Co**	**OR[*]**	**95% CI**	**Ca**	**Co**	**OR[#]**	**95% CI**	**Ca**	**Co**	**OR[^]**	**95% CI**
	2	27	67	1.32	(0.64–2.72)	-	-	-	-	27	67	1.32	(0.64–2.72)
	3	22	70	1.01	(0.48–2.13)	-	-	-	-	22	70	1.01	(0.48–2.13)
			P-trend		0.95						P-trend		0.95
			P-Interaction		0.11						P-interaction		**0.011**

Abbreviations: Cases (Ca), Controls (Co), mitochondrial DNA (mtDNA), Alpha-Tocopherol, Beta-Carotene (ATBC), Prostate, Lung, Colorectal, and Ovarian (PLCO)

[$]Tertiles: PLCO: 1, <99; 2, >99–145; 3, >145; ATBC: 1, <97; 2, >97–121; 3, >121

[*]Adjusted for sex, age, tobacco pack-years, BMI, race, date of blood draw with exception of stratified variable

[#]Adjusted for age, tobacco pack-years, BMI, date of blood draw with exception of stratified variable

[^]Adjusted for sex, age, tobacco pack-years, BMI, race, date of blood draw, cohort with exception of stratified variable

P-trend calculated with the tertiles of mtDNA categories

Table 3

Association of mtDNA copy number and risk of CLL/SLL by cohort and years from blood draw to diagnosis

Cohort		PLCO				ATBC				Pooled			
Subtype	**mtDNA$**	**Ca**	**Co**	**OR***	**95% CI**	**Ca**	**Co**	**OR#**	**95% CI**	**Ca**	**Co**	**OR^**	**95% CI**
Years from Diagnosis to Blood Draw (Males)													
≤6 Years													
	1	9	72	1	(ref)	0	48	-	-	9	120	1	(ref)
	2	11	61	1.49	(0.58–3.88)	3	45	-	-	14	106	1.81	(0.75–4.40)
	3	12	52	1.87	(0.73–4.76)	9	49	-	-	21	101	**3.13**	**(1.35–7.25)**
>6–8 Years			P-trend		0.19						P-trend		0.0078
	1	3	72	1	(ref)	2	48	1	(ref)	5	120	1	(ref)
	2	5	61	2.06	(0.46–9.19)	1	45	0.69	(0.056–8.44)	6	106	1.47	(0.43–5.02)
	3	5	52	2.33	(0.53–10.27)	9	49	**5.72**	**(1.05–31.23)**	14	101	**3.44**	**(1.19–9.99)**
>8 Years			P-trend		0.27		P-trend		**0.019**		P-trend		**0.018**
	1	2	72	1	(ref)	3	48	1	(ref)	5	120	1	(ref)
	2	5	61	2.64	(0.48–14.56)	10	45	3.53	(0.89–13.89)	15	106	**3.08**	**(1.07–8.89)**
	3	5	52	3.36	(0.62–18.22)	10	49	3.16	(0.79–12.61)	15	101	**3.15**	**(1.09–9.11)**
			P-trend		0.16		P-trend		0.13		P-trend		**0.036**
			P-interaction		0.58		P-interaction		0.67		P-interaction		0.65

Abbreviations: Cases (Ca), Controls (Co), mitochondrial DNA (mtDNA), Alpha-Tocopherol, Beta-Carotene (ATBC), Prostate, Lung, Colorectal, and Ovarian (PLCO)

$Tertiles: PLCO: 1, <99; 2, >99–145; 3, >145; ATBC: 1, <97; 2, >97–121; 3, >121

*Polytomous regression: Adjusted for sex, age, tobacco pack-years, BMI, race, date of blood draw

#Polytomous regression: Adjusted for age, tobacco pack-years, BMI, date of blood draw

^Polytomous regression: Adjusted for sex, age, tobacco pack-years, BMI, race, date of blood draw, cohort

P-trend calculated with the tertiles of mtDNA categories