Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study

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Mitochondria are eukaryotic organelles responsible for energy production. Mitochondrial DNA (mtDNA) lacks introns and protective histones, have limited DNA repair capacity and compensate for damage by increasing the number of mtDNA copies. As a consequence, mitochondria are more susceptible to reactive oxygen species, an important determinant of cancer risk, and it is hypothesized that increased mtDNA copy number may be associated with carcinogenesis. We assessed the association of mtDNA copy number and lung cancer risk in 227 prospectively collected cases and 227 matched controls from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, a dose-dependent relationship between mtDNA copy number and lung cancer risk was evident among heavy smokers (n = 229) through 30 April 2002 (11). Fewer than 50% of cases had clear classification as squamous cell carcinoma (n = 74) or adenocarcinoma (n = 34). Controls were selected from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort members who were alive, free of cancer at the time of the case diagnosis, and were individually matched to cases on date of birth (±5 years).

DNA was extracted from the whole blood using the phenol-chloroform method (12), and a fluorescence-based quantitative polymerase chain reaction (QPCR) determined mtDNA copy number (13). Cases and their matched controls were blindly assayed consecutively within each batch. Blinded quality control duplicate samples were interspersed in each batch to evaluate assay reproducibility. The overall coefficient of variation of this assay was 13%. mtDNA copy number data were available for 227 case–control pairs. The correlation between age at randomization and mtDNA copy number was determined by the Spearman correlation coefficient. mtDNA copy number was categorized into quartiles based on the distribution among controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression models, adjusting for age at randomization, number of cigarettes smoked per day and number of years smoking. A test for trend was calculated using mtDNA copy number as a continuous variable. Further adjustment by other factors, such as body mass index, physical activity and caloric intake, did not change the beta-coefficient for mtDNA copy number ≥10%. The mtDNA copy number and smoking interactions were tested by including the interaction term of the dichotomous variables (based on the median among controls) in the model. All statistical analyses were performed using SAS (Cary, NC).

Results

Cases and controls were similar with respect to age, alcohol and energy consumption, physical activity level and trial supplementation group (Table I). As expected, cases smoked more cigarettes per day and smoked for a longer period of time than controls.

Among controls, mtDNA copy number was positively associated with age (r = 0.11, P = 0.08). The median (±standard deviation) mtDNA copy number among cases and controls in the α-tocopherol (cases: 125.6 ± 45.4; controls: 122.2 ± 34.1; P = 0.10) and the β-carotene supplementation arms (cases: 125.6 ± 48.6; controls: 127.4 ± 38.2; P = 0.63) were similar. Men in the highest quartile of mtDNA copy number experienced a significantly increased risk of lung cancer (OR = 2.4; 95% CI = 1.1–5.1) compared with those in the lowest quartile (Table II). There was also evidence that risk of lung cancer increased in a dose-dependent manner with mtDNA copy number (P = 0.008). The association between mtDNA copy number and lung cancer risk was evident among heavy smokers (≥20 cigarettes per day), but not light smokers (<20 cigarettes per day) (Table III); however, the interaction between mtDNA copy number and smoking was not significant. Similar results were seen when classifying heavy and light smokers on the median years of smoking among controls.

Abbreviations: CI, confidence interval; mtDNA, mitochondrial DNA; OR, odds ratio; ROS, reactive oxygen species.

1These authors contributed equally to this work.
Table I. Baseline characteristics of lung cancer cases and individually matched controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 227)</th>
<th>Controls (n = 227)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td>58.7 (5.0)</td>
<td>58.4 (4.8)</td>
<td>0.57</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cigarettes per day</td>
<td>18.8 (9.6)</td>
<td>17.0 (9.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>38.5 (7.1)</td>
<td>35.8 (9.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.6 (3.5)</td>
<td>26.3 (3.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>17.1 (18.5)</td>
<td>18.7 (20.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>Caloric consumption (kcal/day)</td>
<td>2696 (727)</td>
<td>2648 (669)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Physical activity frequency (leisure)
- <1 per week: 103 cases, 106 controls, P-value = 0.79
- 1–2 per week: 73 cases, 76 controls
- ≥3 per week: 51 cases, 45 controls

Supplementation group
- Placebo: 52 cases, 64 controls
- Alpha-Tocopherol: 57 cases, 55 controls
- Beta-Carotene: 58 cases, 55 controls
- Alpha-Tocopherol and Beta-Carotene: 60 cases, 53 controls

*P-values

Table II. ORs and 95% CI for mtDNA copy number and risk of lung cancer

<table>
<thead>
<tr>
<th>mtDNA copy number quartile</th>
<th>ORb, (95% CI)b</th>
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<tbody>
<tr>
<td>Quartile 1 (&lt;100.6)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Quartile 2 (&gt;100.6–124.8)</td>
<td>1.3 (0.7–2.5)</td>
</tr>
<tr>
<td>Quartile 3 (&gt;124.8–151.5)</td>
<td>1.1 (0.6–2.2)</td>
</tr>
<tr>
<td>Quartile 4 (&gt;151.5)</td>
<td>2.4 (1.1–5.1)</td>
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</tbody>
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Table III. mtDNA copy number and risk of lung cancer stratified by smoking level

<table>
<thead>
<tr>
<th>mtDNA copy number</th>
<th>Light smokersa ORb, (95% CI)b</th>
<th>Heavy smokersa ORb, (95% CI)b</th>
<th>P-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;124.79)</td>
<td>1.0 (reference) 43/30</td>
<td>1.6 (0.9–3.0) 41/20</td>
<td>0.37</td>
</tr>
<tr>
<td>High (124.79)</td>
<td>1.1 (0.6–1.9) 75/94</td>
<td>2.6 (1.2–5.5) 57/20</td>
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<td>56</td>
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<tr>
<td>Quartile 3 (&gt;124.8–151.5)</td>
<td>43</td>
<td>57</td>
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<tr>
<td>Quartile 4 (&gt;151.5)</td>
<td>73</td>
<td>57</td>
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*OR and 95% CI determined by conditional logistic regression, adjusted for age at randomization, number of years smoking and number of cigarettes per day.

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*Based on median distribution of number of cigarettes smoked per day in controls (<20 cigarettes per day versus ≥20 cigarettes per day).

To evaluate if undiagnosed lung cancer cases at the time of blood sample collection may have influenced our findings, cases diagnosed within the 2 years of follow-up after blood sample collection were excluded from analyses. After exclusion, the results for the association between mtDNA copy number and lung cancer were similar (P_trend = 0.001). Restriction to only squamous cell carcinoma cases and matched controls yielded similar but non-significant results. Stratified analyses by supplementation arms also yielded similar associations between lung cancer risk and median mtDNA copy number for subjects receiving α-tocopherol (OR = 1.2; 95% CI = 0.4–3.8) or β-carotene (OR = 1.3; 95% CI = 0.4–3.8).

Discussion

Future risk of lung cancer was associated with mtDNA copy number in the present study. Our results suggest that this association may be particularly important among heavy smokers. This is the first study, to our knowledge, to evaluate the risk of lung cancer and mtDNA copy number in a prospective cohort.

Cigarette smoke is a complex mixture of >4000 substances, of which many are chemicals that may introduce high levels of ROS in the human body (14). The ROS burden among smokers is attributed to both oxidants and pro-oxidants found in tobacco smoke (4,5). As such, oxidative stress may play a substantial role in the pathogenesis of smoking-related cancer. Levels of 8-hydroxydeoxyguanosine (8-oxodG), a biomarker for oxidative damage, have been found to be elevated in the lung tissues and peripheral leukocytes of smokers (15,16). Similarly, levels of the oxidative stress biomarker F2-isoprostanes, have also been found to be substantially increased in smokers relative to non-smokers (6–8). Thus, it is conceivable that heavy smokers would have higher internal doses of ROS than light smokers, supporting our findings that heavy smokers would have higher mtDNA copy numbers.

One functional consequence of ROS damage is the disruption of cellular structural elements, including the lipid membranes of mitochondria (17). ROS affect mitochondrial function by damaging mtDNA and impairing electron chain transport. Because mtDNA is in close proximity to the inner membrane of the mitochondria, where electron chain transport occurs, direct oxidative damage to mtDNA is greater than to nuclear DNA. In addition to defenses that scavenge ROS, mitochondria respond to oxidative stress by increasing mtDNA copy number (18–20). In lung tumor tissues, the degree of oxidative mtDNA damage has been found to be significantly associated with mtDNA copy number (21). In fact, mice exposed to tobacco smoke have increased mitochondrial damage compared with unexposed mice (22,23). Thus, our findings that mtDNA copy number is particularly important at predicting future risk of lung cancer among heavy smokers is biologically plausible.

A small case–control study (122 cases; 122 controls) carried out in Xuanwei, China is the only other investigation to have evaluated the risk of lung cancer and mtDNA copy number (24). Similar to our
results, higher mtDNA copy number was associated with lung cancer risk in Xuanwei; however, the retrospective design used in the past study and the evaluation of mtDNA in post-diagnostic biospecimen did not rule out the possibility that the clinical course of lung cancer or treatment might have increased mtDNA copy number in blood of cases compared with controls. It has been hypothesized that the association observed in Xuanwei is attributed to the ROS induced by the high level of inhalation exposures from in-home coal combustion by-products (25). This previous report in Asians, in concert with our findings in Caucasians, suggests that mtDNA may be important to lung cancer etiology.

In conclusion, our results suggest that mtDNA copy number is associated with future development of lung cancer among heavy smokers. The major strength of our study is that the biological samples were collected prospectively, before lung cancer diagnosis. Due to our moderate sample size, replication is needed in larger prospective studies, ideally with more diverse populations (i.e. including women, other races and non-smokers). Future research should also include assays and analyses to elucidate the potential modification of this association by genetic variation of the mitochondrial genome or interindividual variation of mitochondrial function efficiency.

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Acknowledgements
Conflict of Interest Statement: None declared.

References

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