Altered mitochondrial DNA copy number contributes to human cancer risk: evidence from an updated meta-analysis

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Accumulating epidemiological evidence indicates that the quantitative changes in human mitochondrial DNA (mtDNA) copy number could affect the genetic susceptibility of malignancies in a tumor-specific manner, but the results are still elusive. To provide a more precise estimation on the association between mtDNA copy number and risk of diverse malignancies, a meta-analysis was conducted by calculating the pooled odds ratios (OR) and the 95% confidence intervals (95% CI). A total of 36 case-control studies involving 11,847 cases and 15,438 controls were finally included in the meta-analysis. Overall analysis of all studies suggested no significant association between mtDNA content and cancer risk (OR = 1.044, 95% CI = 0.866–1.260, P = 0.651). Subgroup analyses by cancer types showed an obvious positive association between mtDNA content and lymphoma and breast cancer (OR = 1.645, 95% CI = 1.117–2.421, P = 0.012; OR = 1.721, 95% CI = 1.130–2.622, P = 0.011, respectively), and a negative association for hepatic carcinoma. Stratified analyses by other confounding factors also found increased cancer risk in people with drinking addiction. Further analysis using studies of quartiles found that populations with the highest mtDNA content may be under more obvious risk of melanoma and that Western populations were more susceptible than Asians.

Mitochondria are organelles in eukaryotic cells that participate in a wide range of biological actions, such as energy metabolism, free oxygen radical generation, and cell apoptosis. Usually thousands of mitochondria exist in each cell. Mitochondria possess separate copies of DNA (mtDNA), which are double-stranded, maternally inherited circular nucleotides with 16,569 base pairs encoding for 37 RNAs, including 13 mRNA, 22 transfer RNAs and two ribosomal RNAs. mtDNA content in cells typically ranges from 103 to 104 copies per cell and varies by cell type.

Due to the lack of protection from introns and histones, and less efficient DNA repair mechanisms, mtDNA is particularly susceptible to reactive oxidative species (ROS) and other sources of genotoxic damage, which may finally lead to sequence mutations or copy number alterations. Changes in mtDNA copy number could alter the expression of mitochondrial genes and result in abnormal mitochondrial functions, such as energy production, signaling transduction, apoptosis and cell growth. Therefore, aberrant mtDNA content could potentially lead to alterations in oxidative phosphorylation, and enhance the production of ROS in aerobic metabolism, which can cause chromosomal aberrations such as base pair mutation and chromosome rearrangements, and finally facilitate the malignant transformation. Contribution of mtDNA copy number alterations to nuclear genome damage has been confirmed by previous studies. Corral et al. found that introduction of mtDNA molecules to chemically-induced rat hepatomas and HT-1080 fibrosarcoma cell line caused an increased number of copies of COI, COII and COIII pseudogenes in the nuclear genome, while Delsite et al. demonstrated that a reduction in mitochondria in HeLa cells resulted in increased lipid peroxidation. It has been shown that increased lipid peroxidation could induce damage directly to nuclear DNA.

Previous studies have reported quantitative changes in mtDNA content in patients' cancerous tissues compared with their normal counterparts, including an increase of mtDNA content in breast cancer and endometrial

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adenocarcinoma8,9, and a decrease in colorectal cancer, cervical cancer, osteosarcoma and Ewing’s sarcoma10–13. Moreover, the alteration of peripheral blood mtDNA content has also been found to be associated with tumor progression and patient prognosis. For example, lower peripheral blood leukocyte mtDNA copy number was correlated with aggressiveness and fast progression of prostate cancer14. Also, high mtDNA copy number in peripheral blood may predict a poor prognosis for hepatocellular carcinoma15, glioma16, colorectal cancer17, and head and neck cancer18. Taken together, these results suggested that the alteration of mtDNA content might play complicated roles in tumorigenesis and progression in a cancer-specific manner.

As above, the involvement of mtDNA content changes in carcinogenesis remains controversial and ambiguous. Meanwhile, a single study might have been underpowered to detect the overall effects. Thus in this study we carried out a meta-analysis to summarize the existing evidence and to make a more precise evaluation about the relationship between mtDNA copy number and cancer risk among different populations.

Methods

Literature search. Relevant studies that estimated the association between mtDNA copy number and risk of cancers were identified from the databases of PubMed, EMBASE, and China National Knowledge Infrastructure (CNKI) up to March 1, 2016 by two investigators independently. The following key words were used for searching: “mitochondrial DNA”, “risk” and “copy number”. Other alternative spellings of these key words such as “content” and “susceptibility” were also used; only those publications in English were retained.

Inclusion and Exclusion criteria. Studies were considered eligible for the current meta-analysis if they met the following criteria: (1) they assessed the relationship between mtDNA copy number (or mtDNA content) and risk (or susceptibility) of diverse cancer types; (2) used a cohort or case–control studies design; (3) had an appropriate description of mtDNA copy number in cases and controls; and (4) reported an odds ratio (OR) with a 95% confidence interval (CI) or other available data for calculating the OR (95% CI).

Exclusion criteria: (1) review articles and editorials; (2) case reports; (3) studies not focused on the mtDNA content and cancer risk; and (4) investigation of the functional role of mtDNA in carcinogenesis.

Data extraction. The following information from each eligible study was carefully extracted independently by two investigators: name of the first author, year of publication, country of origin, number of cases and controls, numbers of genotyped cases and controls, mitochondrial DNA haplotypes, subject population (categorized as East Asian, Indian, Western and Africa populations), source of controls (hospital-based [HB] and population-based [PB]), exposure to smoking, alcohol consumption, and body mass index (BMI).

Statistical analysis. The strength of the association between mtDNA content and risk of diverse cancers was measured by calculating the odds ratio (OR) with a 95% confidence interval (95% CI), and two-tailed P < 0.05 was considered as statistically significant. First, the overall association between mtDNA content and cancer risk was assessed by categorizing mtDNA content into high and low haplotypes based on cut-off points at the median, the 1st tertile and the 2nd quintile. Specifically, for those studies that categorized the mtDNA content into dichotomies or quartiles, the cut-off point was set at the median value in controls. For those studies categorizing mtDNA into tertiles, the 1st tertile was treated as low mtDNA content group, while the rest were designated as the high content group. The detailed information for the categorization is presented in supplementary Table S1. Nested case-control studies and case-control studies were then combined to calculate the odds ratio in total and separately.

Further association analysis was performed by recruiting studies of quartiles to test if a dose-dependent relationship existed, under which condition the 1st quartile was designated as the reference. Then we compared the odds ratio for the other quartiles with the reference to test whether the higher mtDNA contents correlated with cancer risk more obviously.

Heterogeneity among studies was further examined with the F statistic, which was interpreted as the proportion of total variation contributed by between-study variation. The Mantel–Haenszel method was used to calculate the OR for the pooled data in a fixed effects model if there was no evidence for significant heterogeneity. Otherwise a random effects model was selected to combine the data. Several subgroup meta-analyses were performed in an attempt to assess the association between the mtDNA content and cancer risk based on the population, age, gender, smoking status, alcohol drinking and BMI.

A sensitivity analysis was also performed to examine the reliability of our study by excluding individual studies orderly. Cumulative analysis was performed to explore the tendency of the changes in risk association by publication year. Evidence of publication bias was determined by visual inspection of the funnel plot and Egger’s test. All statistical analyses were performed with STATA software (version 10.0; StataCorp, College Station, TX, USA). All P values are two-sided.

Results

Characteristics of eligible studies. Finally, a total of 36 articles involving 11,847 cases and 15,438 controls were used in the current meta-analysis. By the sample sources according to original records, we classified these 36 articles into 39 independent study subgroups, among which there were twenty-five studies from Western populations, eleven from East Asia, two from India and one from Africa (Table 1). There were five studies with hospital-based controls and the others included population-based controls.

Among these 36 studies, five each focused on breast cancer, four each on renal clear cell carcinoma, three each on lung cancer, colorectal cancer, lymphoma and hepatic carcinoma/cirrhosis, two each on glioma, melanoma, oral and gastric cancer, and one each on nasopharyngeal cancer, endometrial cancer, prostate cancer, esophageal carcinoma, pancreatic carcinoma, soft tissue sarcoma and bladder cancer.
Most studies used ND1 and D-loop which were located on mtDNA as target regions to determine mtDNA copy number. Hemoglobin subunit beta (HBB), human globulin (HGB), 18 s RNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were the most widely used reference genes, and they are widely accepted single copy nuclear genes. All the information about the measurements applied in the studies can be obtained in supplementary Table S1.

![Table 1. Study and host characteristics included in this meta-analysis. Un: Unknown data.](table-data)

| Author       | Year   | Cancer type       | Sample source | Country    | Ethnicity | Sample size Ca/Co | Gender | BMI, kg/m² Ca/Co | Smoking status | Drinking status |
|--------------|--------|-------------------|---------------|------------|-----------|------------------|--------|-----------------|----------------|----------------|}
| Lan          | 2008   | lymphoma          | Population    | Finland    | Caucasian | 104/104          | Male   | 58.5/25.5       | Never          | Un             |
| Shen         | 2010   | Breast cancer     | Population    | USA        | Caucasian | 103/103         | Female | 58.6/26.4       | Ever           | Un             |
| Xing         | 2009   | Renal cell carcinoma | Population   | USA        | Caucasian | 260/281         |        | 52/51           |                |                |
| Bonner       | 2009   | Lung cancer       | Population    | China      | Asian     | 113/107         |        | 75/78           |                |                |
| Hoogood      | 2010   | Lung cancer       | Population    | Finland    | Caucasian | 227/227         |        | 0/0             |                |                |
| Liao         | 2011   | Gastric cancer    | Population    | China      | Asian     | 162/299         |        | Un              |                |                |
| Lymph        | 2011   | pancreatic cancer | Population    | Finland    | Caucasian | 203/656        |        | 0/0             |                |                |
| Qu           | 2011   | Colorectal cancer | Population    | China      | Asian     | 320/320         |        | 126/105         |                |                |
| Zhao         | 2011   | Hepatocellular carcinoma | Population | China      | Asian     | 274/384         |        | 154/132         |                |                |
| Purdue       | 2012   | Renal cell carcinoma | Population   | USA        | Caucasian | 445/379         |        | 293/231         |                |                |
| Thyagarajan  | 2012   | Colorectal cancer | Population    | Singapore  | Chinese   | 422/874         |        | 112/154         |                |                |
| Mondal       | 2012   | Oral cancer       | Hospital      | India      | Asian     | 124/140         |        | 89/75           |                |                |
| Thyagarajan  | 2013   | Breast cancer     | Population    | Singapore  | Chinese   | 183/529         |        | 61/61           |                |                |
| Xie          | 2013   | Soft tissue sarcoma | Population   | USA        | Mixed     | 325/330         |        | 122/117         |                |                |
| Xu           | 2013   | Esophageal adenocarcinoma | Population | USA        | Caucasian | 218/218         |        | 150/122         |                |                |
| Ghosh        | 2014   | Nasopharyngeal carcinoma | Hospital     | India      | Asian     | 64/100          |        | 45/26           |                |                |
| Hoffman      | 2014   | Renal cell carcinoma | Population   | USA        | Caucasian | 230/468         |        | 212/256         |                |                |
| Hosnjeh      | 2014   | Lymphoma          | Population    | European   | Caucasian | 469/469         |        | 272/264         |                |                |
| Huang        | 2014   | Colorectal cancer | Population    | China      | Asian     | 444/1423        |        | 11/16           |                |                |
| Jiang        | 2014   | Breast cancer     | Population    | China      | Asian     | 506/520         |        | 230/240         |                |                |
| Kim ATBC     | 2014   | Lung cancer       | Population    | Finland    | Caucasian | 227/227         |        | 227/227         |                |                |
| Kim PLCO     | 2014   | Population         | Population    | USA        | Mixed     | 426/436         |        | 380/238         |                |                |
| Kim SWHS     | 2014   | Population         | Population    | China      | Asian     | 221/222         |        | 17/11           |                |                |
| Sun          | 2014   | Gastric cancer    | Population    | USA        | Caucasian | 132/125         |        | 55/82           |                |                |
| Zhang        | 2014   | Glioma            | Population    | China      | Asian     | 414/414         |        | 96/85           |                |                |
| Zhou         | 2014   | Prostate cancer   | Population    | China      | Asian     | 193/194         |        | 100/98          |                |                |
| Hyland       | 2014   | Melanoma          | Population    | USA        | Mixed     | 136/302         |        | 97/93           |                |                |
| Kim ATBC     | 2015   | Lymphoma          | Population    | Finland    | Caucasian | 142/142         |        | 142/142         |                |                |
| Kim PLCO     | 2015   | Lymphoma          | Population    | USA        | Mixed     | 292/301         |        | 135/135         |                |                |
| Lemnrau      | 2015   | Breast cancer     | Population    | UK         | Caucasian | 1053/1053       |        | 428/410         |                |                |
| Melkonian    | 2015   | Renal cell carcinoma | Population   | USA        | Caucasian | 608/629         |        | 297/287         |                |                |
| Shen         | 2015   | Breast            | Population    | USA        | Caucasian | 1000/1000       |        | 720/750         |                |                |
| Shen         | 2016   | Melanoma          | Population    | USA        | Mixed     | 500/500         |        | 280/250         |                |                |
| He           | 2014   | Oral premalignant lesion | Population  | USA        | Mixed     | 143/357         |        | 83/175          |                |                |
| Sun          | 2015   | Endometrial cancer | Hospital      | USA        | Caucasian | 139/139         |        | 85/108          |                |                |
| Wang         | 2015   | Liver Cirrhosis   | Hospital      | USA        | Caucasian | 136/136         |        | 68/60           |                |                |
| Williams     | 2015   | Bladder cancer    | Hospital      | USA        | Caucasian | 926/926         |        | 67/52           |                |                |
| Hashad       | 2015   | Liver carcinoma/Cirrhosis | Population | Egypt      | African   | 90/45           |        | 198/204         |                |                |
| Shen         | 2016   | Glioma            | Population    | USA        | Caucasian | 395/425         |        | 195/216         |                |                |
Association between mtDNA content and cancer risk in overall and stratified analyses. When all studies were pooled for the overall meta-analysis by dichotomizing mtDNA copy number into high and low content groups, there was no significant association between mtDNA content and overall cancer risk (OR = 1.044, 95% CI 0.866–1.260, P = 0.651), and there was high heterogeneity (I² = 92.4%), which may be explained by different cancer types and populations (Fig. 1).

Results of the subgroup analyses are presented in Table 2. Stratification analysis by cancer types identified increased cancer risk associated with high mtDNA content in the subgroups of breast cancer (OR = 1.721, 95% CI = 1.130–2.622, p = 0.011) and lymphoma (OR = 1.645, 95% CI = 1.117–2.421, p = 0.012 respectively), with high and moderate heterogeneity, respectively (I² = 92.6% and 51.6%). Marginal increased risk associated with high mtDNA content was found in melanoma and glioma, with an odds ratio of 1.555 (95% CI = 0.598–4.045, p = 0.365, I² = 93.6%) and 2.510 (95% CI = 0.709–8.887, p = 0.154, I² = 97.2%), respectively. In contrast, mtDNA content was negatively significantly correlated with hepatic malignancy (OR = 0.622, 95% CI = 0.486–0.798, p = 0.000). We further categorized the studies by organ systems, such as digestive, respiratory, gynecological and urinary carcinoma. However, no significant association was identified in any group.

Further subgroup analyses were performed by stratifying studies according to population, age, and gender, as well as life styles such as smoking status, alcohol consumption and body mass index (Table 2). The results revealed that high mtDNA content may predict increased carcinogenesis risk in people with drinking addition (OR = 1.261, 95% CI = 1.076–1.479, p = 0.004). A total of five studies that provided drinking-related data were included for stratified analysis, and it is notable that four of these studies came from digestive cancers, indicating that high mtDNA content accompanied by drinking addiction may contribute to elevated digestive cancer risk.
According to the study design, we classified the studies into nested case-control and case-control studies (supplementary Table S1), and we performed association analysis based on the study design as well (Table 3). The results from the nested case-control and case-control study did not differ significantly from the results above, which demonstrated the stability and reliability of our analysis. It was surprising to find that mtDNA content was negatively significantly correlated with renal cancer risk in the case-control studies, which was obscured in the stratified analysis in the previous section. Cancer risk increased as mtDNA content elevated by quartile analyses. To investigate the possible dose-dependent relationship between mtDNA content and cancer risk, we enrolled the studies that categorized mtDNA copy number into quartiles for further analysis and assigned the 1st quartile as the reference. A total of 20 studies were included in the dose-dependent relationship analyses. No significant difference was found in the risk among carriers of the 2nd, 3rd and 4th quartiles in the overall analysis. However, stratified analysis by cancer types found that carriers of higher mtDNA content exhibited gradually increasing risk for breast cancer and melanoma (Table 4), which is consistent with the findings in the dichotomous meta-analysis, further supporting the idea that high mtDNA predisposes increased risk in breast cancer and melanoma. After grouping studies by population, the association of mtDNA content in 4th quartiles with cancer risk was more obvious in western populations than other populations. 

| Subgroups | Research counts (n) | Test of association | Heterogeneity |
|-----------|--------------------|---------------------|---------------|
|           |                    |                     |               |               |               |               |               |
| Overall   | 39                 | Random              | 1.044         | 0.866-1.260   | 0.651         | 499.08        | 0.000         | 92.4%         | 0.3139        |
| Cancer types |                   |                     |               |               |               |               |               |               |               |
| Lymphoma  | 4                  | Random              | 1.645         | 1.117-2.421   | 0.012*        | 6.20          | 0.102         | 51.6%         | 0.0769        |
| Breast cancer | 5                | Random              | 1.721         | 1.130-2.622   | 0.011*        | 54.16         | 0.000         | 92.6%         | 0.2015        |
| Lung cancer | 5                 | Random              | 0.982         | 0.788-1.223   | 0.871         | 7.84          | 0.097         | 49.0%         | 0.2015        |
| Colorectal cancer | 3         | Random              | 1.168         | 0.638-2.139   | 0.641         | 34.38         | 0.000         | 94.2%         | 0.2681        |
| Renal carcinoma | 4       | Random              | 0.856         | 0.598-1.226   | 0.397         | 19.71         | 0.000         | 84.8%         | 0.1126        |
| Melanoma  | 2                  | Random              | 1.555         | 0.598-4.045   | 0.365         | 15.73         | 0.000         | 93.6%         | 0.456        |
| Glioma    | 2                  | Random              | 2.510         | 0.709-8.887   | 0.154         | 35.96         | 0.000         | 97.2%         | 0.8091        |
| Gastric   | 2                  | Random              | 0.412         | 0.054-3.134   | 0.392         | 25.76         | 0.000         | 96.1%         | 2.0587        |
| Hepatic   | 3                  | Fixed               | 0.622         | 0.486-0.798   | 0.000¹        | 0.53          | 0.767         | 0.0%           |               |
| Oral      | 2                  | Random              | 0.946         | 0.295-3.029   | 0.925         | 12.43         | 0.000         | 92.0%         | 0.6489        |

| Organ systems |               |                     |               |               |               |               |               |               |               |
| Digestive system |         | 12                 | Random         | 0.861         | 0.632-1.173   | 0.344         | 95.74         | 0.000         | 88.5%         | 0.2521        |
| Urogenital system |       | 6                 | Random         | 0.876         | 0.635-1.209   | 0.420         | 43.03         | 0.000         | 88.4%         | 0.1398        |
| Respiratory system |       | 6                 | Random         | 0.928         | 0.730-1.179   | 0.539         | 11.74         | 0.039         | 57.4%         | 0.0488        |
| Gynecologica system |     | 6                 | Random         | 1.253         | 0.744-2.109   | 0.396         | 106.55        | 0.000         | 95.3%         | 0.3847        |

| Population |               |                     |               |               |               |               |               |               |               |
| Western    | 25               | Random              | 0.951         | 0.719-1.259   | 0.728         | 456.55        | 0.000         | 94.7%         | 0.4628        |
| East Asian | 11               | Random              | 1.296         | 0.901-1.866   | 0.162         | 147.15        | 0.000         | 93.2%         | 0.3481        |
| India      | 2                | Fixed               | 0.508         | 0.335-0.771   | 0.001         | 0.01          | 0.938         | 0.0%           |               |
| Africa     | 1                |                     |                | 0.799         |               |               |               |               |               |

| Age⁰      |               |                     |               |               |               |               |               |               |               |
| Elder     | 10              | Random              | 1.104         | 0.655-1.860   | 0.710         | 111.22        | 0.000         | 91.9%         | 0.6352        |
| Younger   | 10              | Random              | 1.079         | 0.670-1.738   | 0.754         | 95.11         | 0.000         | 90.5%         | 0.5213        |

| Gender    |               |                     |               |               |               |               |               |               |               |
| Male      | 16              | Random              | 1.036         | 0.755-1.420   | 0.828         | 155.98        | 0.000         | 90.4%         | 0.3578        |
| Female    | 22              | Random              | 1.109         | 0.866-1.420   | 0.414         | 191.26        | 0.000         | 89.0%         | 0.2788        |

| Life styles |           |                     |               |               |               |               |               |               |               |
| Smoking status |         |                     |               |               |               |               |               |               |               |
| Ever/Frequent | 21       | Random              | 0.984         | 0.765-1.267   | 0.902         | 106.17        | 0.000         | 81.2%         | 0.2465        |
| Never/Occasionally | 18 | Random              | 1.047         | 0.736-1.491   | 0.798         | 165.82        | 0.000         | 89.7%         | 0.4972        |

| Drinking Status |         |                     |               |               |               |               |               |               |               |
| Ever/Frequent | 5          | Fixed               | 1.261         | 1.076-1.479   | 0.004⁴        | 4.53          | 0.340         | 11.6%         |               |
| Never/Occasionally | 5  | Random              | 1.179         | 0.971-1.432   | 0.096         | 42.69         | 0.000         | 90.6%         | 0.5403        |

| BMI         |               |                     |               |               |               |               |               |               |               |
| High⁰     | 4              | Random              | 1.103         | 0.413-2.942   | 0.845         | 36.84         | 0.000         | 91.9%         | 0.9038        |
| Low⁰      | 4              | Random              | 1.077         | 0.550-2.109   | 0.828         | 15.12         | 0.002         | 80.2%         | 0.3609        |

Table 2. Overall and subgroup analyses of mtDNA copy number with cancer risk by dichotomizing mtDNA copy number into high and low groups. ¹p < 0.05; ²Age was classified as elder and younger according to publication records; ³Relative value which means relatively high and relatively low BMI.
in Asians (OR = 1.122, p = 0.016 versus OR = 0.981, p = 0.896). There were no obvious differences in cancer risk among males and females or among frequent and infrequent smokers.

**Cumulative analysis.** The findings from the cumulative meta-analysis showed that the positive association was more obvious in early studies but decreased as the data increased by publication year, and the association strength remained stable in the 95% confidence intervals with increasing sample sizes in recent years (Fig. 2), which further demonstrated the complicated roles of mtDNA content in diverse carcinogenesis.

**Sensitivity analyses.** A sensitivity analysis was conducted to assess the influence of each study by sequential omission of each eligible study both in the overall meta-analysis and stratified analysis. The results showed that the significance of the OR was not affected by any single study (Fig. 3), indicating the reliability of our study.

**Evaluation of publication bias.** Both Begg's funnel plot and Egger's test were performed to assess the publication bias. The shape of the funnel plots did not reveal any evidence of obvious asymmetry for any genetic model in the overall meta-analysis (Fig. 4). Next, Egger's test was used to provide statistical evidence for the funnel plot symmetry, and the results still did not suggest any obvious evidence of publication bias (P = 0.868). Thus, there was no obvious risk of publication bias in this meta-analysis.

**Discussion**
Mitochondria are energy factories that produce adenosine triphosphate (ATP) through oxidative phosphorylation. Both increased and decreased mtDNA copy number in blood have been previously reported to be associated with cancer risk in a cancer-specific manner. For example, it has been reported that increased mtDNA copy number contributes to high risk for breast cancer and lymphoma, while decreased mtDNA content was relevant to renal and colorectal cancers. The opposite effects brought out by altered mtDNA content may represent diverse biological roles that mtDNA plays in malignancies.

Biologically, lowered mtDNA copy number can cause deficiency in oxidative phosphorylation and enhanced production of toxic metabolites in aerobic metabolism and glycolysis, resulting in the disruption of cellular functions.

| Subgroups               | Research counts (n) | Test of association Model | OR        | 95% CI    | P        | Heterogeneity I² | P        | F | Tau- squared |
|-------------------------|---------------------|---------------------------|-----------|-----------|----------|------------------|----------|---|--------------|
| Overall                 |                     |                           |           |           |          |                  |          |   |              |
| Nested case-control     | 16                   | Random                    | 1.135     | 0.957-1.342 | 0.651 | 62.08 | 0.000 | 75.8% | 0.0816      |
| Case-control            | 23                   | Random                    | 0.958     | 0.708-1.297 | 0.783 | 436.96 | 0.000 | 95.0% | 0.5011      |
| Cancer types            |                     |                           |           |           |          |                  |          |   |              |
| Lymphoma*               | 4                    | Random                    | 1.645     | 1.117-2.421 | 0.012* | 6.20  | 0.102 | 51.6% | 0.0769      |
| Breast cancer           | 2                    | Fixed                     | 1.360     | 1.165-1.587 | 0.000* | 1.77  | 0.183 | 43.6% | 0.4645      |
| Breast cancer b         | 3                    | Random                    | 2.001     | 0.897-4.468 | 0.090 | 46.98 | 0.000 | 95.7% | 0.4645      |
| Lung cancer             | 4                    | Random                    | 0.888     | 0.752-1.049 | 0.163 | 2.29  | 0.514 | 0.0%  | 0.2015      |
| Lung cancer             | 1                    |                           |           |           |          |                  |          |   |              |
| Colorectal cancer       | 2                    | Random                    | 0.891     | 0.760-1.045 | 0.156 | 14.68 | 0.000 | 93.2% | 0.6489      |
| Colorectal cancer       | 1                    |                           |           |           |          |                  |          |   |              |
| Renal carcinoma         | 1                    |                           |           |           |          |                  |          |   |              |
| Renal carcinoma         | 3                    | Fixed                     | 0.711     | 0.611-0.827 | 0.000* | 0.15  | 0.926 | 0.0%  | 0.4456      |
| Melanoma                | 2                    | Random                    | 1.555     | 0.598-4.045 | 0.365 | 15.73 | 0.000 | 93.6% | 0.2015      |
| Glioma                  | 2                    | Random                    | 2.510     | 0.709-8.887 | 0.154 | 35.96 | 0.000 | 97.2% | 0.8091      |
| Gastric                 | 2                    | Random                    | 0.412     | 0.054-3.134 | 0.392 | 25.76 | 0.000 | 96.1% | 2.0587      |
| Hepatic                 | 1                    |                           |           |           |          |                  |          |   |              |
| Hepatic                 | 2                    | Fixed                     | 0.630     | 0.472-0.842 | 0.002* | 0.50  | 0.479 | 0.0%  | 0.0%        |
| Oral                    | 2                    | Random                    | 0.946     | 0.295-3.029 | 0.925 | 12.43 | 0.000 | 92.0% | 0.6489      |
| Organ systems           |                     |                           |           |           |          |                  |          |   |              |
| Digestive system        | 5                    | Random                    | 0.920     | 0.673-1.258 | 0.602 | 20.22 | 0.204 | 80.2% | 0.0975      |
| Digestive system        | 7                    | Random                    | 0.711     | 0.399-1.266 | 0.247 | 75.74 | 0.000 | 92.1% | 0.5452      |
| Urogenital system       | 1                    |                           |           |           |          |                  |          |   |              |
| Urogenital system       | 5                    | Random                    | 0.808     | 0.637-1.025 | 0.079 | 16.11 | 0.003 | 75.2% | 0.0533      |
| Respiratory system      | 4                    | Random                    | 0.888     | 0.752-1.049 | 0.163 | 2.29  | 0.514 | 0.0%  | 0.2015      |
| Respiratory system      | 2                    | Random                    | 0.942     | 0.279-3.181 | 0.924 | 7.97  | 0.004 | 87.4% | 0.6742      |
| Gynecological system    | 2                    | Fixed                     | 1.360     | 1.165-1.587 | 0.000* | 1.77  | 0.183 | 43.6% | 0.8848      |
| Gynecological system    | 4                    | Random                    | 1.143     | 0.443-2.949 | 0.782 | 103.65| 0.000 | 97.1% | 0.8848      |

Table 3. Comparison of cancer risk association between nested case-control and case-control studies. aThe bold inclined letters indicate the nested case-control study. bThe thin inclined letters indicate the case-control study. cP < 0.05.
Also, it has been demonstrated that mtDNA reduction promotes cancer cells to become more resistant to apoptosis, and leads to epithelial-mesenchymal transition (EMT)\textsuperscript{19,20}, an important process by which epithelial cells lose their cell polarity and cell-cell adhesion and acquire mesenchymal, fibroblast-like characteristics, accompanied by increased cell migratory and invasive properties, thus endowing the incipient cancer cell with invasive and metastatic properties\textsuperscript{21}. On the other hand, high mtDNA content can be an indicative marker of oxidative stress, which is associated with environmental exposure to pollutants, tobacco, smoke, drugs, xenobiotics, or radiation. High mtDNA content is also an important marker of an impaired aerobic mechanism that is thought to be involved in the molecular mechanisms of carcinogenesis\textsuperscript{22}. These observations together suggest a complicated role for the changes in mtDNA copy number on the modulation of cancer risk. In the present study, we performed this meta-analysis to achieve a more conclusive result.

Our analysis included 36 studies that focused on diverse cancer types from different origins around the world\textsuperscript{23–58}. To reduce the uncertainty induced by confusing factors in the meta-analysis, we evaluated the association between mtDNA content with cancer risk in an overall analysis, as well as stratified analyses by cancer types, genders, ages, ethnicity and life styles.

Finally, we did not find an obvious association between mtDNA content and cancer risk in the overall analysis, which should be the result of the heterogeneity among different cancer types. Thus subgroup analyses were performed to further detect the association strength. Stratified analysis by cancer types provided evidence on the relationship between high mtDNA content and increased breast cancer and lymphoma risk. Dose-dependent effects were also found for melanoma risk. The biological contribution of increased mtDNA content in the peripheral blood to carcinogenesis remains obscured. Previous studies found a correlation between mtDNA content and markers of oxidative stress, such as thiobarbituric acid reactive substances and 8-hydroxyguanosine\textsuperscript{59}. Moreover, lower levels of antioxidants were also detected in blood with an increased mtDNA copy number. Due to lack of sufficient protective mechanisms, mtDNA mutations may occur during the process of oxidative stress, and certain mtDNA mutations may lead to the generation of increased superoxide and nitric oxide, resulting in aberrant mitochondrial biogenesis\textsuperscript{60}, which has been associated with deficient or defective apoptosis, and confers a replicative advantage to the cells\textsuperscript{61}. Therefore it is plausible that high mtDNA content in peripheral blood leukocytes may be indicative of increased oxidative stress, impaired aerobic metabolism, and ROS-mediated DNA damage. mtDNA copy number may increase to compensate for mtDNA damage and mitochondrial dysfunction\textsuperscript{62}. Thus it is more likely that the increased mtDNA content is a significant marker of carcinogenesis associated oxidative stress, but not a cause of cancer development.

Stratified analysis also found a significant negative association between mtDNA content and susceptibility of hepatic carcinoma, suggesting the complexity of the involvement of mtDNA changes in carcinogenesis.
in different populations, which should not be explained by any single mechanism. It has been demonstrated that mtDNA decrease alters mitochondrial gene expression, resulting in deficiency in oxidative phosphorylation, and causes a disturbance of cellular functions. Warburg proposed that damage to the respiratory chain was a critical event in carcinogenesis, which demonstrates that cancer cells enhanced the generation of adenosine triphosphate by glycolysis, resulting in a stronger tolerance to hypoxia and reducing the dependence of mitochondrial oxidative phosphorylation, thus conferring tumor cells the advantages of growth. Enhanced glycolysis also leads to excessive production of lactate and prosurvival proteins, which may initiate and promote cancer development. MtDNA reduction was also found to increase cancer cells’ resistance to apoptosis and lead to epithelial-mesenchymal transition, which are both common in tumor formation and metastatic progression. Some previous studies have partially explained the mechanism by which mtDNA reduction acts on apoptosis resistance and epithelial-mesenchymal transition. Biswas et al. reported that decreased mtDNA content could activate NFκB/Rel factors. Activation of nuclear factor-kappa B signaling plays a critical role in apoptosis resistance and is clearly linked to various cancer malignant transformation. In addition, activation of the AKT pathway by an mtDNA deficiency could also inhibit cell apoptosis. As for the contribution to EMT, mtDNA loss may activate PI3K/Akt2, and Raf/MAPK pathways, which may finally lead to EMT and cancer metastasis.

Figure 2. Cumulative analysis of the overall ORs. The results were sorted by publication years.
phosphorylated by mitochondria, so we proposed that abnormal mtDNA content may predict altered cancer risk in the smoking population. However, we did not obtain the suspected results, indicating that tumor-genesis of smoking related cancers involves much more cellular abnormality than mtDNA changes.

In summary, our study provides evidence that increased mtDNA content is statistically significantly associated with risk of lymphoma, breast cancer and melanoma, but is negatively associated with hepatic carcinoma. The mechanism for the tumor specific associations between mtDNA content and cancer risk remain to be illustrated, although they are likely to be regulated by a wide range of genetic, molecular, and cellular determinants. For instance, elevated mtDNA content has been significantly associated with altered oxidative stress, aging, immune response activation, and response to environmental exposure. Future studies are warranted to evaluate oxidative stress related factors that influence mtDNA copy number and to provide novel insights into the biological

Figure 3. Sensitivity analysis of the summary ORs on the association between mtDNA content and overall cancer risk. The results were calculated by omitting each eligible study. Meta-analysis random-effects estimates were used.

Figure 4. Begg's funnel plot for publication bias analysis. Each point represents a separate study for the indicated association. Log[or], natural logarithm of the odds ratio. Horizontal line indicates effect size.
mechanisms of mtDNA copy number variation on the development of various cancers, especially on the opposite effects of increased and decreased mtDNA content in carcinogenesis. Furthermore, many more subjects are needed to confirm the associations identified by our meta-analysis in different populations.

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