Mitochondrial Common Deletion, a Potential Biomarker for Cancer Occurrence, Is Selected against in Cancer Background: A Meta-Analysis of 38 Studies

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Abstract

Mitochondrial dysfunction has long been proposed to play a major role in tumorigenesis. Mitochondrial DNA (mtDNA) mutations, especially the mtDNA 4,977 bp deletion has been found in patients of various types of cancer. In order to comprehend the mtDNA 4,977 bp deletion status in various cancer types, we performed a meta-analysis composed of 33 publications, in which a total of 1,613 cancer cases, 1,516 adjacent normals and 638 healthy controls were included. When all studies were pooled, we found that cancerous tissue carried a lower mtDNA 4,977 bp deletion frequency than adjacent non-cancerous tissue (OR = 0.43, 95% CI = 0.20–0.92, P = 0.03 for heterogeneity test, I² = 91.5%) among various types of cancer. In the stratified analysis by cancer type the deletion frequency was even lower in tumor tissue than in adjacent normal tissue of breast cancer (OR = 0.19, 95% CI = 0.06–0.61, P = 0.005 for heterogeneity test, I² = 82.7%). Interestingly, this observation became more significant in the stratified studies with larger sample sizes (OR = 0.70, 95% CI = 0.58–0.86, P = 0.0005 for heterogeneity test, I² = 95.1%). Furthermore, when compared with the matched healthy controls, increased deletion frequencies were observed in both adjacent non-cancerous tissue (OR = 3.02, 95% CI = 2.13–4.28, P < 0.00001 for heterogeneity test, I² = 53.7%), and cancerous tissue (OR = 1.36, 95% CI = 1.04–1.77, P = 0.02 for heterogeneity test, I² = 83.5%). This meta-analysis suggests that the mtDNA 4,977 bp deletion is often found in cancerous tissue and thus has the potential to be a biomarker for cancer occurrence in the tissue, but at the same time being selected against in various types of carcinoma tissues. Larger and better-designed studies are still warranted to confirm these findings.

Introduction

Mitochondria are ubiquitous organelles in eukaryotic cells with the primary function to generate energy in the form of ATP through the coupling of oxidative phosphorylation (OXPHOS) [1]. Mitochondria contain their own genome. In human, mitochondrial genome is a 16.5 kb compactly organized, double-stranded, and closed molecule [2]. Human mtDNA contains 37 genes encoding 13 polypeptides, all of which are the components of the respiratory chain/OXPHOS system, 2 ribosomal RNAs and 22 transfer RNAs [2,3]. Due to the lack of a sophisticated DNA repair mechanism, mtDNA is more prone to attacks by reactive oxygen species (ROS), a byproduct of respiration, and the somatic mutation rate of mtDNA is presumed to be 10 to 20 times higher than that of nuclear DNA [4,5].

Defects in mitochondrial function have long been hypothesized to play a role in tumorigenesis [6], and a large number of mtDNA mutations have been detected in a variety of cancers [7,8]. Reported sequence changes in cancer patients including point mutations, multiple deletions and microsatellite instability (MSI) in coding and noncoding regions [9–12]. One of the best-described mtDNA mutation is the mtDNA 4,977 bp or common deletion [13], which deletes between nucleotides 8,470 and 13,447 of the human mtDNA. This mutation removes all or part of the genes encoding four complex I subunits, one complex IV subunit, two complex V subunits and five tRNA genes, which are indispensable for maintaining normal mitochondrial function [14].

The mitochondrial common deletion has attracted tremendous interests as it is associate with several sporadic diseases including myopathies, Alzheimer disease, Pearson’ s syndrome, photaging of the skin, Kearns-Sayre syndrome (KSS) and chronic progressive external opthalmoplegia (CPEO) [5,13,15–19]. Furthermore, this deletion also accumulates in many tissues during aging, and has been used as an indication of mtDNA oxidative damage [20,21].
Although many studies associate this common deletion with tumorigenesis, results from population studies remain conflicting rather than conclusive [22–24]. Several studies have found the mtDNA 4,977 bp deletion in various types of cancer, including in cancer of the breast, endometrium, esophagus, stomach, neck, liver, lung, oral, kidney, skin and thyroid [22,23]. In some cases, the incidence and level of the 4,977 bp deletion were lower in the cancer tissue compared with adjacent non-cancerous tissue from the same patients [24]. In some reports no significant associations were detected [25]. Mitochondrial DNA deletions serve as biomarkers of aging in the skin, but are typically absent in non-melanoma skin cancers. Thus, the role of this common deletion in tumorigenesis is intriguing, but largely perplexing, and each of these single studies may have been underpowered to detect the association between mtDNA common deletion and cancers because of their small sample size. In the hope that the underlying heterogeneity among different studies can be resolved in a large scale analysis, we conducted a systematic meta-analysis on 33 relevant published articles with 1613 cases, 1516 adjacent normals and 630 healthy controls to drive a more precise estimation, and with an improved statistic power to detect the association of this mtDNA alteration with various cancer types.

Materials and Methods

Search Strategy and Selection Criteria
We carried out a search in Pubmed and other public domains with a combination of the following keywords: ‘4,977 bp deletion’, ‘cancer’ or ‘carcinoma’, and ‘risk’ to identify all case-control studies published to date on an association between mitochondrial DNA 4,977 bp deletion and various types of cancer (last search update in November, 2012). Additional studies were identified by a hands-on search of bibliographies of original studies on this topic.

Studies were included if they meet the following criteria: full-text articles in English; case-control design study; odds ratios (ORs) or confidence intervals (CIs) (or, if 95% CIs were not reported, the reported data were sufficient to calculate them); if more than one article was published using the same patient population, only the latest or the complete study would be used in this analysis. We excluded studies if the crucial data were not reported in original papers, or if they had a very high probability of inaccurate reporting.

Data Extraction
Data were independently extracted by two authors (Hezhong-rong Nie and Hongying Shua) and checked by the other author, any disagreement was resolved by discussions until consensus was reached. The following information was extracted from all included publications: the first author, year of publication, cancer types, total number of cases and controls, country and ethnicity of the study population, DNA source, number of included patients, deletion detection method and deletion results of each study. For studies including subjects of different racial descents, data were extracted separately for each ethnic group categorized as European ancestry (EA), Asian or others. When a study did not state the ethnic group type or if it was impossible to separate participants according to the data presented, the sample was termed as ‘others’. Furthermore, references involving different cancer type, different ethnic group, different sample size, different DNA source and different measurement method were divided into multiple study samples for subgroup analyses.

Quantitative Data Synthesis
The numbers of cases and controls by deletion status from each study were collected to evaluate this deletion frequency among various types of cancer (ORs and 95% CIs), meanwhile, the median of the relative deletion level were also collected from some studies who displayed the data (data not shown). Furthermore the stratification analyses were also conducted by ethnicity (EA or Asian), cancer type (if one cancer type was investigated in less than two studies, it would be merged into the ‘other cancers’ group), sample size (not larger than 50 and larger than 50), measurement method and DNA source.

Statistical Analysis
Heterogeneity was quantified with the $I^2$-based Q test and $I^2$ statistic, using Q test to assess between-study heterogeneity and considered significant if $P$<0.05 [26], and $I^2$ statistic can work out a value that indicates what proportion of the total variation across studies is beyond chance. Specifically, 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity [27]. The choice of fixed-effects model or the random-effects model was based on the Mantel-Haenszel method and the DerSimonian and Laird method. When $I^2$ value of the heterogeneity test was<0.05, the fixed-effects model was used, which assumes the same homogeneity of effect size across all studies [28]. Otherwise, the random-effects model was more appropriate, which tends to provide wider confidence intervals as the results of the constituent studies differ among themselves [29]. Subgroup analyses were also performed by ethnic group, cancer type, sample size, measurement method and DNA source. To assess the effects of individual studies, sensitivity analysis was performed by excluding each study at a time individually and recalculating the ORs and confidence intervals. Potential publication bias was estimated by the inverted funnel plot, in which the standard error of log (OR) of each study was plotted against its log (OR) [30], and an asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was assessed by Begg’s and Egger’s linear regression test. The significance of the intercept was determined by the $t$ test as suggested by Egger, and $P$<0.05 was considered representative of statistically significant publication bias [30]. This meta-analysis was performed by using the software Review Manage (v.4.2) and Stata 12.0 (Stata Corporation, College Station, TX). All the $P$-values were two-sided, and a $P$<0.05 was considered statistically significant.

Results

Study Characteristics
A total of 768 potentially relevant records were identified by using the key words mentioned earlier in the Methods, of which 50 were examined the mtDNA 4,977 bp deletion frequency in cancers after the title and abstract review. After full-text review, 17 were excluded because of several reasons such as they did not provide available data or the studies were reviews. Finally, a total of 38 studies in 33 articles met our inclusion criteria. The characteristics of each case-control study are listed in Table 1. Of the 38 studies, sample sizes ranged from 7 to 130, in which nine studies focusing on breast cancer [25,31–38], five studies focusing on hepatocellular carcinoma [39–43], four focusing on gastric cancer [38,44–46], three focusing on colorectal cancer [25,38,47], three focusing on esophageal cancer [48–50], three focusing on oral cancer [51–53], two focusing on thyroid cancer [25,54], two focusing on skin cancer [55,56] and seven studies of other cancers (one endometrial carcinoma [57], one lung cancer [58], one Warthin’s cancer [59], one cervix cancer [60], one acute
lymphoblastic leukemia [61], one prostate cancer [62] and one head and neck cancer [39]). Because some controls in two researches [25,38] were shared by several cancers, it was defined as four studies (breast cancer, colorectal cancer, gastric cancer and head and neck cancer) and three studies (thyroid cancer, breast cancer and colorectal cancer) in the analysis stratified by cancer type but defined as one study in the overall analysis and stratification analysis by ethnicity, sample size, measurement method and DNA source. Overall, 12 studies used EA, 20 used Asians and one used other ethnic groups. These were 27 case/adjacent normal studies and 15 case/healthy normal studies included. Additionally, the tissue was the most common source of DNA, although other sources were also applied, such as blood and

| Surname, year | Country | Ethnicity | Cancer type | Group 1 | Group 2 | Group 3 | DNA source | Measurement method |
|---------------|---------|-----------|-------------|---------|---------|---------|------------|-------------------|
| Kamalidehghan, 2006 | Iran | Asian | gastric | 6/107 | 18/107 | tissue | regular PCR |
| Tan, 2009 | UK | EA | esophageal | 2/12 | 9/10 | 1/12 | tissue | regular PCR |
| Bianchi, 1995 | Argentina | EA | breast | 1/7 | 3/7 | tissue | regular PCR |
| Aral, 2010 | Turkey | EA | thyroid, breast and colorectal | 4/100 | 1/100 | 0/49 | tissue and blood | regular PCR |
| Chen, 2011 | China | Asian | colorectal | 17/104 | 13/104 | tissue | regular PCR |
| Abnet, 2004 | China | Asian | esophageal | 17/19 | 19/20 | tissue and blood | regular PCR |
| Dai, 2006 | China | Asian | lung | 20/37 | 22/37 | 6/20 | tissue | regular PCR |
| Dani, 2004 | Brazil | others | breast, colorectal, gastric and head and neck | 43/87 | 74/87 | 2/17 | tissue and blood | regular PCR |
| Gwak, 2011 | Italy | EA | hepatocellular | 3/27 | 24/27 | 8/8 | tissue | regular PCR |
| Kamenisch, 2007 | Germany | EA | skin | 37/41 | 40/41 | tissue | regular PCR |
| Kara, 2012 | Turkey | EA | cervix | 4/21 | 5/16 | tissue | regular PCR |
| Futyma, 2008 | Poland | EA | endometrial | 30/37 | 32/37 | tissue | regular PCR |
| Lee, 2001 | Taiwan | Asian | oral | 26/53 | 36/53 | tissue | regular PCR |
| Lewis, 2000 | UK | EA | Warthin’s tumor | 14/14 | 6/6 | tissue | qPCR |
| Rahul, 2012 | India | Asian | oral | 42/50 | 18/50 | tissue | regular PCR |
| Upadhyay, 2009 | India | Asian | esophageal | 2/39 | 1/39 | tissue | regular PCR |
| Shao, 2004 | China | Asian | hepatocellular | 19/27 | 12/27 | tissue | qPCR |
| Fukushima, 1994 | Japan | Asian | hepatocellular | 7/28 | 23/35 | tissue | regular PCR |
| Tan, 2003 | Taiwan | Asian | oral | 2/18 | 5/18 | tissue | regular PCR |
| Tan, 2012 | USA | EA | breast | 0/19 | 0/19 | tissue | regular PCR |
| Tseng, 2006 | Taiwan | Asian | breast | 3/60 | 28/60 | tissue | regular PCR |
| Tseng, 2009 | Taiwan | Asian | breast | 3/60 | 29/60 | tissue | regular PCR |
| Maximo, 2002 | Porto | EA | thyroid | 26/44 | 9/42 | tissue | regular PCR |
| Pavicic, 2009 | Argentina | EA | breast | 43/95 | 70/95 | 78/199 | tissue | regular PCR |
| Wang, 2009 | China | Asian | gastric | 86/108 | 73/108 | 29/56 | tissue | regular PCR |
| Wen, 2011 | China | Asian | ALLg | 26/26 | 39/39 | blood | qPCR |
| Wheelhouse, 2005 | China | Asian | hepatocellular | 17/62 | 59/62 | 9/9 | tissue | regular PCR |
| Wu, 2005 | Taiwan | Asian | gastric | 3/31 | 17/31 | tissue | regular PCR |
| Yang, 2004 | Taiwan | Asian | skin | 12/17 | 26/53 | tissue | regular PCR |
| Ye, 2008 | China | Asian | breast | 76/76 | 76/76 | tissue | qPCR |
| Yin, 2004 | Taiwan | Asian | hepatocellular | 18/18 | 18/18 | tissue | regular PCR |
| Yu, 2010 | China | Asian | prostate | 98/130 | 14/130 | tissue | regular PCR |
| Zhu, 2004 | USA | EA | breast | 18/39 | 13/39 | 6/23 | tissue | regular PCR |

*Acute lymphoblastic leukemia.
*a-deletion number.
*total number.
*tissue.
*Quantitative PCR.
*Non-cancerous tissue.
*Healthy normal tissue.
* Fellow of the National Natural Science Foundation of China.
buccal swab [25,32,38,48,49]. Buccal swab was termed as tissue in the stratified analysis by DNA source.

Meta Analysis Results

We obtained the mtDNA 4977 bp deletion data from 33 publications consisting of 1613 cases, 1516 adjacent normal and 638 healthy controls. When all eligible studies were pooled into the meta-analysis, we found that cancerous tissue carried a lower mtDNA 4,977 bp deletion frequency than adjacent non-cancerous tissue (OR = 0.43, 95% CI = 0.20–0.92, \( P = 0.03 \) for heterogeneity test, \( I^2 = 91.5\% \); Fig. 1A). However, no significant association was found if the samples were divided into EA subject group (OR = 0.43, 95% CI = 0.11–1.73, \( P = 0.23 \) for heterogeneity test, \( I^2 = 91.4\% \)) or Asian subject group (OR = 0.49, 95% CI = 0.17–1.44, \( P = 0.20 \) for heterogeneity test, \( I^2 = 93.3\% \)). In the stratified analysis by cancer type (Table 2), we found that the difference of detection of 4,977 bp deletion in cancerous samples and corresponding non-cancerous breast samples was more pronounced (OR = 0.19, 95% CI = 0.06–0.61, \( P = 0.005 \) for heterogeneity test, \( I^2 = 82.7\% \); Fig. 1B); but this phenomenon did not exist in hepatocellular cancer (OR = 0.10, 95% CI = 0.00–3.74, \( P = 0.21 \) for heterogeneity test, \( I^2 = 93.4\% \)), gastric cancer (OR = 0.33, 95% CI = 0.06–1.67, \( P = 0.18 \) for heterogeneity test,

![Figure 1. Odds ratios (ORs) and 95% confidence intervals (CIs).](doi:10.1371/journal.pone.0067953.g001)
I² = 86.9%), esophageal cancer (OR = 0.28, 95% CI = 0.02–3.73, P = 0.34 for heterogeneity test, I² = 69.0%) and colorectal cancer (OR = 0.66, 95% CI = 0.37–1.16, P = 0.15 for heterogeneity test, I² = 87.6%).

As breast cancer has been the most studied one with the implication of mitochondrial dysfunction and mtDNA in tumor-igenesis, we examined the role of sample size in the detection of the effect of common deletion. Interestingly, we found the difference in deletion frequency in cancer tissue and in adjacent non-cancerous tissue was becoming more significant when the sample size is larger than 50 (OR = 0.70, 95% CI = 0.58–0.86, P = 0.0005 for heterogeneity test, I² = 95.1%; Fig. 2A). As for the experimental methods, the regular PCR appeared to be more sensitive (OR = 0.39, 95% CI = 0.18–0.86, P = 0.02 for heterogeneity test, I² = 91.7%; Fig. 2B).

To further determine if mtDNA common deletion could serve as a potential marker for tumor-igenesis, we then compared the frequencies of common deletion detection in cancer patient, cancerous tissue and adjacent non-cancerous tissues with tissues from the healthy controls, we found it was more likely to detect mtDNA common in tissues from the cancer patients in both cancerous (OR = 1.36, 95% CI = 1.04–1.77, P = 0.02 for heterogeneity test, I² = 83.5%; Fig. 3A) and adjacent non-cancerous (OR = 3.02, 95% CI = 2.13–4.28, P<0.0001 for heterogeneity test, I² = 53.7%; Fig. 3B) tissues.

### Sensitivity Analyses

We performed sensitivity analysis to assess the stability of the results of this meta-analysis by sequentially excluding each study in both case/adjacent normal and case/healthy normal groups. The leave-one-out sensitivity analysis indicated that no single study changed the pooled ORs qualitatively, suggesting the stability of this meta-analysis (Fig. S1A and Fig. S1B).

### Publication Bias

Publication bias was assessed by Begg’s funnel plot and Egger’s test. As shown in Fig. S2A and Fig. S2B, the shapes of the funnel plots seemed symmetrical in both case/adjacent normal and case/healthy normal groups, suggesting the absence of publication bias. Meanwhile, the Egger’s test was performed to provide statistical evidence of funnel plot asymmetry. The results indicated no significant evidence for publication bias of the current meta-analysis (P = 0.081 in case/adjacent normal group and P = 0.573 in case/healthy normal group). All together, we believe that bias from publications might not have a significant influence on the results of our meta-analysis.

### Discussion

In this meta-analysis of 1613 cancer cases, 1516 adjacent normals and 638 healthy controls from 33 independent publications, while the mtDNA 4,977 bp deletion was found in both cancerous and adjacent normal tissue, when compared to matched healthy controls, the deletion frequency was significantly lower in tumor tissue than in adjacent non-cancerous tissue among various types of cancer. This indicates that the mtDNA 4,997 bp deletion is selected against in cancerous tissue as compared to the adjacent normal tissue, making it an attractive biomarker for cancer occurrence.

Mitochondrial DNA is continuously exposed to oxidative stress, thus accumulating a large load of mutations, one of the most common being the mtDNA 4,977 bp deletion [22]. The mtDNA 4,977 bp deletion has been reported in several kinds of cancers, such as breast cancer [31,34,35,38], colorectal cancer [38], gastric cancer [38,45,46], hepatocellular carcinoma [42,43], lung cancer [58], head and neck cancer [38], esophageal cancer [63] and thyroid carcinoma [64]. Also not surprisingly, due to the low

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**Table 2.** The mtDNA 4977 bp deletion status in stratification analysis by selected factors.

| Stratification | No. of studies | OR(95%CI) | P<sub>het</sub><sup>a,b</sup> | No. of studies | OR(95%CI) | P<sub>het</sub><sup>b,c</sup> |
|----------------|---------------|-----------|-----------------|---------------|-----------|-----------------|
| **Ethnicity**  |               |           |                 |               |           |                 |
| Caucasian      | 10            | 0.40 (0.13–1.25) | 0.11 | 7 | 0.97 (0.34–2.78) | 0.95 |
| Asian          | 16            | 0.49 (0.17–1.44) | 0.2 | 6 | 0.96 (0.16–5.66) | 0.97 |
| **Cancer type** |               |           |                 |               |           |                 |
| Breast cancer  | 9             | 0.19 (0.06–0.61) | 0.005 | 4 | 0.44 (0.28–0.70) | 0.0005 |
| Gastric cancer | 4             | 0.33 (0.06–1.67) | 0.18 | 1 | – | – |
| Colorectal cancer | 3<sup>d</sup> | – | – | – | – | – |
| Esophageal cancer | 3 | 0.28 (0.02–3.73) | 0.34 | – | – | – |
| Hepatocellular cancer | 4 | 0.10 (0.00–3.74) | 0.21 | 3<sup>e</sup> | – | – |
| **Sample size** |               |           |                 |               |           |                 |
| ≤50            | 15            | 0.46 (0.18–1.19) | 0.11 | 10 | 1.09 (0.32–3.64) | 0.89 |
| >50            | 12            | 0.70 (0.58–0.86) | 0.0005 | 5 | 1.60 (1.13–2.27) | 0.009 |
| **Method**     |               |           |                 |               |           |                 |
| Regular PCR    | 25            | 0.39 (0.18–0.86) | 0.02 | 13 | 1.36 (0.62–2.97) | 0.44 |
| qPCR           | 2             | – | – | 2 | – | – |

*Case/adjacent normal group.

<sup>a</sup>P value of the Q-test for heterogeneity test.

<sup>b</sup>Case/healthy normal group.

<sup>c</sup>One study was excluded.

<sup>d</sup>Couldn't define its heterogeneity.

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specificity of this common deletion, mtDNA 4,977 bp deletion has also been shown to be implicated in the occurrence of various types of degenerative diseases and aging [43,65,66] as well as in healthy infants and children [67]. Both animal studies and cell model analysis showed the mtDNA 4,977 bp deletion played an important role during the course of tumorigenesis [68,69]. However, the results of various studies dissecting the role of this mutation in cancer development are conflicting. For example, the study of Ye found that though all of their samples carry mtDNA 4,977 bp large deletion, they thought there was no correlation between mtDNA 4,977 bp common deletion and cancer risk [34]; Tseng et al demonstrated that the detection frequency of common deletion was more higher in adjacent normal tissue than carcinoma tissue in their 60 breast cancer patients from Taiwan [35]; meanwhile, the analysis of Dani concurred with the results from Tseng in several cancer types [38]. The mtDNA 4,977 bp common deletion mutations is a highly non-specific mutation and as mentioned before has been seen in degenerative disorders as well as in healthy tissues. This can account for the inconsistency in the data obtained so far. Clearly, other cellular as well as tissue

Figure 2. Odds ratios (ORs) and 95% confidence intervals (CIs) for various cancer types associated with mtDNA 4977 bp deletion in case/adjacent normal group. (A) sample size larger than 50. (B) regular PCR measurement method.
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specific environmental factors may also affect the outcome of this mutation on the cell survival and tumorigenesis. As for the proportion of this deletion in cancer tissues, it may be influenced by many factors, including its presence in cancer precursor cell[s] and the growth rate for cancer cells. Hence, while a promising observation, future studies are required to determine whether the mutation can be used as a successful biomarker for cancer. Our previous studies have shown that heteroplasmic DNA mutation, where the burden of mutation is less, was more tumorigenic than homoplasmic mutation where all mtDNA is mutated [70]. Our studies have also shown a difference in the retrograde signaling pathways between homoplasmic mitochondria and heteoplasmic mitochondria with lesser load of a particular mutation activating survival pathways involving ROS and Akt thus making cells tumorigenic while inhibition of tumorigenesis was seen in cells with homoplasmic mutations. This supports our hypothesis that a larger mutation load may be required during early stages to tumor development, the mutation being then selected against if the cells have to continue the proliferative growth leading to a greater frequency of mutations in adjacent normal tissue as compared to cancerous tissue. The mtDNA 4,977 bp deletion can thus serve as a potential biomarker for early stage tumor development. The inconsistent results may also due to the individual studies with small sample sizes and thus not enough statistical power to detect the reliable association. Therefore, to deal with the problem of sample size and to understand the role of this mutation in cancer, we conducted this meta-analysis where we found that the mtDNA 4,977 bp deletion were less frequent in various cancer tissues as compared with adjacent non-cancerous tissues. Although this meta-analysis showed the mtDNA common deletion was more likely happening in different types of adjacent non-cancerous tissues than in cancerous tissues, some results from stratification analysis remind us of drawing the conclusion with caution. The stratified analysis by cancer type showed that the mtDNA 4,977 bp deletion was more likely in adjacent non-cancerous tissue of breast cancer patients ($P = 0.005$), which contains the largest sample size in our cancer type stratified analysis. Our heterogeneity analysis showed that cancer type did contribute to substantial heterogeneity, these inconsistent results by cancer types may involve different carcinogenic mechanisms. One possible explanation for the differences found between different non-cancerous tissue was the existence of tissue-specific mtDNA turnover rates and various environmental and genetic influences. Different biological pathways (such as repair of oxidative damage, metabolism of hormone and age-related cell signal pathway) could interact with mtDNA, resulting in different

![Figure 3. Odds ratios (ORs) and 95% confidence intervals (CIs) for various cancer types associated with mtDNA 4977 bp deletion.](A) case/healthy normal group. (B) adjacent normal/healthy normal group of tissue sample stratified analysis. doi:10.1371/journal.pone.0067953.g003)
efforts on mtDNA damage and cancer susceptibility. For example, several studies found the effect of mtDNA 4,977 bp deletion on colorectal cancer was significant for certain subgroups, such as patients under 65 years old were more likely to carry this deletion in tumor tissues ($P = 0.0027$ [47]). This result indicated that there is possibly a negative selection for the common deletion in tumor tissues during aging [47].

Furthermore, the significantly lower mtDNA 4,977 bp deletion frequency in cancer tissue compared with adjacent non-cancerous tissue of larger sample size studies (OR = 0.39, 95% CI = 0.38–0.86, $P = 0.0005$ for heterogeneity test, $I^2 = 95.1%$) suggests to us that genetic risk of cancer conferred by the common variants was very modest and the penetrance of the variants was very small, which means that even if the variation was crucial for carcinogenesis, extremely large-scale evidence would be necessary to establish with high confidence the presence of specific associations.

However, the results from stratification analysis by measurement method indicated that the tumor tissue deletion frequency was also lower than the adjacent non-cancerous tissue in studies with the detection method of regular PCR (OR = 0.39, 95% CI = 0.18–0.86, $P = 0.02$ for heterogeneity test, $I^2 = 91.7%$). But we considered the statistical power was limited in the analyses of such subgroups, these studies suffer from several major drawbacks, such as information bias, selection bias, lower study size and inferior statistical power, which may have a substantial influence on the results of our meta-analysis. Considering those limitations included in the stratified analysis, our results should be interpreted with caution since it may not have enough statistic power. In measurement method stratification analysis, only two studies using quantitative PCR in case/adjacent normal group and case/healthy normal group, so we couldn’t carry out the heterogeneity analysis. In this meta-analysis, although regular PCR was more frequently used for the detection of 4,977 bp deletion compared with quantitative PCR, quantitative PCR was more convenient and less time consume.

Therefore, the findings of mtDNA common deletion frequency in different cancers in this meta-analysis still require further replication with more precise analysis and larger studies to avoid the drawbacks.

Some limitations of the current study should be addressed. First, the total number of studies was too small to perform further subgroup analyses. Second, in the subgroup analysis by ethnicity, the included studies involved only EA and Asians, data concerning other ethnic groups such as Africans were not found. Third, only publications in English selected by databases were included in this meta-analysis. It is possible that some relevant publications or unpublished studies or publications in other languages were missed, which might place a bias on our results. Further, there is some evidence that age and cigarette smoke can alter the mtDNA 4,977 bp deletion frequency [47,50]. However, due to the lack of original data in the publications, we didn’t stratify the studies by these factors. More accurate OR should be corrected by age, gender, smoking status, alcohol consumption and other exposure factors.

In conclusion, this study was the first meta-analysis to assess the association between mtDNA 4,977 bp deletion and cancers, and this paper provided evidence that mtDNA 4,977 bp deletion is more likely to happen in cancer patient, but is selected against in various types of cancerous tissues. However, due to the limitations of original studies included in the meta-analyses, further larger-scale studies using appropriate methods and subjects are required to evaluate the role of mtDNA 4,977 bp deletion in cancer development. In addition, such studies should also take environmental factors into account, so as to gain a better and more comprehensive understanding of unraveling the underlying mechanism of mtDNA 4,977 bp deletion in cancer development and progression.

Supporting Information

Figure S1 Sensitivity analysis of studies. (A) case/adjacent normal group. (B) case/healthy normal group. (TIF)

Figure S2 Funnel plot analysis to detect publication bias. (A) case/adjacent normal group. (B) case/healthy normal group. (TIF)

Author Contributions

Conceived and designed the experiments: HN JL YB. Performed the experiments: HN HS YM XH HF LS ZD. Analyzed the data: HN HS RV AM JL YB. Wrote the paper: HN RV AM YB.

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