Published in final edited form as:

Mol Carcinog. 2015 October ; 54(10): 1220–1226. doi:10.1002/mc.22189.

Detection of DNA Damage in Peripheral Blood Mononuclear Cells from Pancreatic Cancer Patients

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Abstract

DNA repair is a key mechanism in maintaining genomic stability: repair deficiencies increase DNA damage and mutations that lead to several diseases, including cancer. We extracted DNA from peripheral blood mononuclear cells (PBMCs) of 48 pancreatic adenocarcinoma cases and 48 healthy controls to determine relative levels of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) damage by QPCR. All participants were never smokers and between the ages of 60 and 69. Average levels among cases were compared to controls using a rank sum test, and logistic regression adjusted for potential confounding factors (age, sex, and diabetes mellitus). Cases had less DNA damage, with a significant decrease in mtDNA damage (p-value = 0.03) and a borderline significant decrease in nDNA damage (p = 0.08). Across samples, we found mtDNA abundance was higher among non-diabetics compared to diabetics (p = 0.04). Our results suggest that patients with pancreatic adenocarcinoma have less DNA damage in their PBMCs, and that having diabetes, a known pancreatic cancer risk factor, is associated with lower levels of mtDNA abundance.

DNA repair has long been thought to be a key mechanism in maintaining genomic stability[1]. Deficient DNA repair has been associated with increased risk of mutations, cellular dysfunction, and DNA damage that can lead to cellular senescence, neurodegeneration, aging, and cancer[2–5]. Research on polymorphisms in genes involved in DNA damage repair and risk of developing pancreatic cancer has yielded significant associations with several genes[6–8], suggesting an association between DNA repair and pancreatic carcinogenesis.

Some investigators have used a systems approach to identify the role that defective DNA repair plays in cancer, such as assays that measure total DNA damage[9]. Unfortunately

these assays often are labor intensive or require fresh samples, which are often difficult to obtain[9]. An assay using the quantitative polymerase chain reaction (QPCR) technique is versatile because of it can be performed using small amounts of stored samples and has rapid turnaround time. The QPCR assay result provides a relative measure of amplification where any form of damage inhibiting DNA polymerase will reduce the measured amplification[10,11]. Therefore, decreased amplification of a target sequence relative to a control sample is indicative of greater DNA damage. Typically, this type of damage results from endogenous reactive oxygen species or exogenous genotoxins that generate a variety of DNA lesions such as base modifications, abasic sites, and single and double strand breaks. These DNA lesions are especially damaging because they lead to point mutations, insertions/deletions, and genomic rearrangements[12,13]. Using the QPCR technique, we can obtain a relative measure of DNA damage in both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) as well as a relative measure that is proportional to mtDNA abundance. Moreover, the assay detects DNA damage that has accumulated over time as well as recent DNA damage that has yet to be repaired[11]. This assay has been successfully used for the study of human molecular epidemiology using peripheral blood[9].

There are no studies investigating DNA damage (either among nDNA or mtDNA) in peripheral blood of pancreatic cancer cases compared to controls. Only one published cohort study of 203 incident cases and 656 control smoking males investigated pancreatic cancer and mtDNA copy number, and demonstrated an increased average copy number measured by real-time PCR[14]. The mitochondrion functions as a regulator of cell life and death [15–17] and its DNA is much more prone to oxidative damage than nDNA for several reasons as presented in Basso et al.[18]. It has been hypothesized that increased mtDNA copy number is a mechanism the cell uses to deal with increased damage to the mtDNA[19–21].

DNA damage is thought to accumulate over time, as older age groups have significantly greater DNA damage compared to younger groups[2–5]. Additionally, type 2 diabetics have been shown to exhibit increased levels of DNA damage compared to controls[22,23]. Since pancreatic cancer incidence also increases with age and is associated with diabetes, we hypothesized that increased accumulation of DNA damage over time plays a significant role in pancreatic carcinogenesis, and that secondarily; type 2 diabetics will also have an increased amount of DNA damage in their peripheral blood mononuclear cells compared to non-diabetics.

Methods

Sample

This study was approved by the Mayo Clinic Institutional Review Board. Both cases and controls were selected from an existing Biospecimen Resource for Pancreas Research. Under that protocol, all persons with suspected pancreatic cancer or related disease were recruited, provided informed consent, questionnaire data, and blood samples, typically prior to initiation of any treatment regimen. Controls were recruited from the Department of Internal Medicine and frequency matched to cases on age, gender, and region of residence. Participants were selected to be never smokers and between ages of 60–69 to avoid confounding issues between these variables and DNA damage when testing our hypotheses.

Estimation of nuclear DNA damage, mitochondrial DNA damage, and mitochondrial DNA abundance by quantitative PCR (QPCR)

The QPCR assay was performed as previously described with some modifications [24]. Briefly, DNA from peripheral blood mononuclear cells (PBMCs) was isolated using QIAamp DNA isolation kit from Qiagen. The concentration of DNA was determined by using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen). PCR reactions were performed using 7.5 ng of target DNA for amplification of the small mtDNA amplicon and 10 ng for the amplification of the large mtDNA amplicon as well as the nuclear amplicon, 10 pmol/μl of oligonucleotide primers and 0.5 units of the MasterAmp™ Extra-Long DNA Polymerase (Epicentre). PCR products were quantified using PicoGreen and visualized by 1% agarose (large amplicon; see below) or 15% polyacrilamide (small amplicon; see below) gel electrophoresis. Gels were stained with ethidium bromide and visualized under UV-light. Preliminary experiments were performed to ensure optimal PCR buffer conditions using commercially available PCR premixes (Epicentre) and to determine the optimal number of PCR cycles.

To determine relative mtDNA abundance, we amplified a 124 bp human mitochondrial fragment (small mitochondrial amplicon). Since the probability of finding DNA lesions in such a small fragment is very low, changes in the amounts of PCR products will be representative of changes in mtDNA abundance. The amplification profile for the small mitochondrial amplicon was as follows: an initial denaturation for 45 sec at 94°C; followed by 23 cycles of denaturation for 15 sec, annealing/extension at 68°C for 12 min. A final extension at 72°C was performed for 10 min. The primer nucleotide sequences used for the amplification of the human small mitochondrial amplicon were the following: 5'-CAT GCA AGC ATC CCC GTT CC-3' (sense) and 5'-CTG TTT CCC GTG GGG GTG TG-3' (antisense). PCR reactions were carried out using Premix 3. Relative mtDNA abundance was expressed as a ratio $A_{\text{Cases}}/A_{\text{Controls}}$, where A_{Cases} is the amount of amplification product (small mtDNA amplicon) from pancreatic cancer patients and A_{Controls} is the amount of amplification product (small mtDNA amplicon) from Controls. Values represent the average of three independent PCR reactions.

To determine relative levels of damage to the mitochondrial genome, we amplified a human mitochondrial fragment that was approximately 6.9 kbp (large mitochondrial amplicon) using the following PCR amplification profile: an initial denaturation for 45 sec at 95°C; followed by 23 cycles of denaturation for 15 sec, annealing/extension at 68°C for 12 min. A final extension at 72°C was performed for 10 min. The primer nucleotide sequences used for the amplification of the human large mitochondrial amplicon were the following: 5'-CCC AAG GCA CCC CTC TGA CA-3' (sense) and 5'-GCC CGT GGG CGA TTA TGA GA -3' (antisense). PCR reactions were carried out using Premix 1. mtDNA damage was expressed as a ratio ($A_{\text{Cases}}/A_{\text{Controls}}$) using the amplification of the large mtDNA amplicon, which was previously corrected for possible variations in mtDNA abundance using the small mtDNA amplicon. Values represent the average of three independent PCR reactions.

Relative levels of nuclear DNA damage were determined by PCR amplification of a human nuclear fragment approximately 6.9 kbp as follows: an initial denaturation for 45 sec at 95°C; followed by 29 cycles of denaturation for 15 sec, annealing/extension at 68°C for 12

min. A final extension at 72°C was performed for 10 min. The primer nucleotide sequences used for the amplification of the human large mitochondrial amplicon were the following: 5'-TTG AGA CGC AT GAGA CGT GCA G-3' (sense) and 5'-TCA CAT TCT TGG CTG GGT GTG G-3' (antisense). The PCR reactions were carried out using Premix 1. PCR products were quantified as above described and the amplification was expressed relative to a reference DNA. Values represent the average of two independent PCR reactions.

Statistical analyses

For each DNA damage measure, we compared the values in cases of pancreatic adenocarcinoma compared to those in controls using a rank sum test. We also evaluated continuous measures as variables in a logistic regression model to calculate odds ratios (OR) and 95% confidence intervals, adjusting for potential confounding variables including age, sex, and diabetes mellitus. All tests were determined to be significant at $p = 0.05$. Analyses were generated using SAS® software (Version (9.2))[25].

Results

We applied QPCR to measure levels of DNA damage in PBMCs from pancreatic cancer patients and healthy controls. The QPCR assay has been used by us and other research groups to successfully detect DNA damage in a variety of cells and tissues[24,26–29]. By comparing the relative amplification of a damaged DNA template versus an undamaged template, it is possible to estimate levels of DNA damage.

In our sample of 48 cases and 48 controls, a greater proportion of cases were female, diabetic and exhibited a slightly higher participant-reported usual body mass index (BMI). The age at the time of recruitment and the alcohol intake were similar, with all participants selected based on an age range of 60–69 and reported to never have smoked cigarettes (Table 1).

Table 2 compares the mean and 95% confidence interval and median with range for our square root transformed measures of mtDNA damage, mtDNA abundance and nDNA damage in cases and controls. Based on the nonparametric rank sum test, cases have significantly less damage in their mtDNA (higher average square root transformed mtDNA amplification)($p=0.03$). There is also a statistically borderline indication that cases have a lower amount of damage in their nDNA (higher average square root transformed nDNA amplification)($p=0.08$). Table 2 shows logistic regression results with odds ratios (ORs) and 95% confidence intervals for models, both unadjusted and adjusted for age, sex, and type 2 diabetes mellitus (T2DM). None of these models reached statistical significance.

In Table 3, we examined DNA damage based on self-reported T2DM. The nonparametric rank sum test is statistically significant and provides evidence that diabetics compared to non-diabetics have lower median mtDNA abundance (higher average square root transformed small mtDNA amplification $p = 0.04$).

Discussion

In this study of DNA from PBMCs of 96 subjects, we found that pancreatic adenocarcinoma cases have lower levels of mtDNA damage (Table 2; $p = 0.03$). Similarly, nDNA damage was lower in cases although the statistical significance was borderline ($p = 0.08$). No significant differences in levels in mtDNA abundance were detected between cases and controls. In a sub-analysis among our entire sample, we observed that type 2 diabetic subjects have less mtDNA abundance compared to non-diabetics (Table 3, $p = 0.04$). These results suggest that PBMCs from pancreatic cancer patients exhibit less DNA damage than PBMCs from control subjects.

There are several plausible explanations for the observation of decreased mtDNA damage in PBMCs from pancreatic cancer. First, pancreatic tumors may induce a general state of oxidative stress, which may result in increased DNA repair capacity PBMCs, thus decreasing the levels of basal DNA damage in PBMCs. Evidence for such a phenomenon has recently been obtained in animals bearing nonmetastatic tumors which exhibited a systemic inflammation state characterized by DNA damage in some distal tissues [30]. The induction of DNA repair capacity by oxidative stress is well documented [31]. Thus, pancreatic cancer-dependent oxidative stress might be inducing DNA repair capacity in PBMCs leading to decreased basal levels of DNA damage. We have recently reported a similar effect in PBMCs from middle age rhesus macaques, which show increased markers of oxidative stress in several tissues concomitantly with decreased levels of mtDNA damage in PBMCs [24].

A second explanation for the observation of decreased mtDNA damage in PBMCs from pancreatic cancer is that increased mtDNA damage in PBMCs may lead to apoptosis of those cells in which damage reaches a threshold, thus reducing the number of damaged cells within the pool of circulating lymphocytes. Experiments on normal human fibroblasts expressing human telomerase reverse transcriptase and in lung endothelial cells show that oxidative stress can induce persisting mtDNA damage which can lead to mitochondrial dysfunction followed by apoptosis [32,33].

Lastly, the changes in mtDNA abundance detected in our study could be due to decreased number of white blood cells, particularly leukocytes. The levels of mtDNA in leukocytes are particularly sensitive to ROS[34]. Since most pancreatic cancer require adjuvant chemotherapy after surgery[Campan, 2011 #160] it is not uncommon that these patients exhibit leucopenia. Further studies are required to determine the impact of specific white blood cell subpopulations in mtDNA abundance and damage in pancreatic cancer.

In a case-control study nested in the Beta-Carotene Cancer Prevention (ATBC) Study cohort of male smokers, Lynch et al.[14] reported a significantly higher mtDNA copy number associated with pancreatic cancer. In our study, we did not observe a statistically significant difference among pancreatic adenocarcinoma cases compared to controls. Possible explanations for this discrepancy may be that our sample consisted of male and female nonsmokers (compared to Lynch's study of only smokers), or smaller sample size. A technical discrepancy between the method used in our study (relative mtDNA abundance)

and that used by Lynch et al. (absolute mtDNA quantification) is unlikely since our method has been shown to detect increases in mtDNA[35]. In general, across and within numerous cancer sites regardless of DNA source, there have been inconsistent results regarding measured mtDNA copy number and these inconsistencies may be due to numerous factors such as sample source and methodology employed[36–40].

A connection between mitochondrial dysfunction and pancreatic adenocarcinoma is suggested by repeated observations that a higher proportion of cases have type 2 diabetes compared to those without cancer[41]. A few investigators have attempted to determine if mtDNA repair and/or mtDNA copy number are associated with pancreatic cancer and if there is a potential link with diabetes. Tyrberg et al. [42] supports a model where hyperglycemia and consequent increased beta-cell oxidative metabolism leads to DNA damage and induction of Ogg1 expression, a protein active in DNA repair within the mitochondria. The review by Basso et al. [18] concluded that mitochondrial dysfunction leads to development of pancreatic adenocarcinoma. In our study, we observed that mtDNA abundance was significantly higher (Table 4, $p = 0.04$) among diabetics compared to non-diabetics, based on the nonparametric rank sum test. However, when comparing cases and controls using the logistic regression model adjusted for diabetes, age, and sex (Table 2), we did not observe a difference compared to the unadjusted logistic model, suggesting minimal information gained with diabetes in the model after knowing case status. Also, we cannot disregard that the limited number of diabetic cases and controls may have affected our comparison.

To the authors' knowledge there are no studies that examined basal DNA damage in the PBMCs of pancreatic cancer patients or in pancreatic cancer tissue compared to controls. In our study, we found a borderline significantly higher average DNA amplification in cases compared to controls ($p = 0.08$), suggesting lower nDNA damage among cases. Interestingly, a study on PanIN lesions, which are thought to be precursors to pancreatic cancer, has demonstrated increasing DNA damage with increasing PanIN classification (–1, –2, –3) with higher levels at each grade compared to pancreatic ductal epithelium[43]. Although this study did not specifically look at mtDNA damage and abundance, it suggests that pronounced DNA damage may be an early event during tumor progression. For other cancers, previous studies many of which use the COMET assay (reviewed in Collins et al., 2008 [44]) have generally shown that DNA damage is higher among cases, including non-melanoma skin cancer,[45] head and neck cancer,[46] lung cancer,[47] and breast cancer[48–52].

We selected the QPCR assay to study DNA damage in PBMCs because it provides several advantages over other techniques, such as host cell reactivation assay or the comet assay[53,54]. Also, this approach has been successfully used to detect mtDNA damage in peripheral blood from Frederick ataxia patients, demonstrating the use of peripheral blood as a surrogate tissue for certain diseases and highlighting the role of mtDNA damage in systemic pathologies[9]. However, there are also limitations of the QPCR assay, which include the inability to specifically identify the lesions blocking the PCR polymerase, and the method provides a relative, rather than absolute measure of DNA damage. Recent evidence suggests that the DNA extraction method affects telomere length measurements.

[34] Our selection of the QIAamp isolation kit may affect comparison between our observed DNA damage measurements and those of other studies using different extraction methods.

In conclusion, this study suggests that in PBMC DNA from pancreatic adenocarcinoma cases there is lower DNA damage compared with controls. Specifically, we observed that cases had lower amounts of mtDNA damage and marginally lower levels of nDNA damage. We found that having diabetes, a known pancreatic cancer risk factor, was associated with lower levels of mtDNA abundance. These results would suggest that among subgroups there is reduced DNA damage.

Acknowledgements

We thank all the study participants and the pancreatic cancer research team members for their contributions to the study, including Robert R. McWilliams, M.D..

Partial funding for this research was provided by G12RR003051, G12MD007600, U54 CA096297, R25TCA92049, and Mayo Clinic SPORE in Pancreatic Cancer (P50 CA102701).

References

1. Garm C, Moreno-Villanueva M, Burkle A, et al. Genetic and environmental influence on DNA strand break repair: A twin study. *Environ Mol Mutagen*. 2013
2. Jeppesen DK, Bohr VA, Stevnsner T. DNA repair deficiency in neurodegeneration. *Prog Neurobiol*. 2011; 94(2):166–200. [PubMed: 21550379]
3. Wolters S, Schumacher B. Genome maintenance and transcription integrity in aging and disease. *Front Genet*. 2013; 4:19. [PubMed: 23443494]
4. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *Journal of the National Cancer Institute*. 2000; 92(11):874–897. [PubMed: 10841823]
5. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability--an evolving hallmark of cancer. *Nature reviews Molecular cell biology*. 2010; 11(3):220–228. [PubMed: 20177397]
6. Jiao L, Hassan MM, Bondy ML, et al. XRCC2 and XRCC3 gene polymorphism and risk of pancreatic cancer. *The American journal of gastroenterology*. 2008; 103(2):360–367. [PubMed: 17986315]
7. Duell EJ, Holly EA, Bracci PM, Wiencke JK, Kelsey KT. A population-based study of the Arg399Gln polymorphism in X-ray repair cross- complementing group 1 (XRCC1) and risk of pancreatic adenocarcinoma. *Cancer research*. 2002; 62(16):4630–4636. [PubMed: 12183419]
8. Jiao L, Bondy ML, Hassan MM, et al. Selected polymorphisms of DNA repair genes and risk of pancreatic cancer. *Cancer detection and prevention*. 2006; 30(3):284–291. [PubMed: 16844323]
9. Haugen AC, Di Prospero NA, Parker JS, et al. Altered gene expression and DNA damage in peripheral blood cells from Friedreich's ataxia patients: cellular model of pathology. *PLoS Genet*. 2010; 6(1):e1000812. [PubMed: 20090835]
10. Ayala-Torres S, Chen Y, Svoboda T, Rosenblatt J, Van Houten B. Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. *Methods*. 2000; 22(2):135–147. [PubMed: 11020328]
11. Santos JH, Meyer JN, Mandavilli BS, Van Houten B. Quantitative PCR-based measurement of nuclear and mitochondrial DNA damage and repair in mammalian cells. *Methods Mol Biol*. 2006; 314:183–199. [PubMed: 16673882]
12. Campen CJ, Dragovich T, Baker AF. Management strategies in pancreatic cancer. *Am J Health Syst Pharm*. 2011; 68(7):573–584. [PubMed: 21411798]
13. Wyman C, Kanaar R. DNA double-strand break repair: all's well that ends well. *Annu Rev Genet*. 2006; 40:363–383. [PubMed: 16895466]

14. Lynch SM, Weinstein SJ, Virtamo J, et al. Mitochondrial DNA copy number and pancreatic cancer in the alpha-tocopherol beta-carotene cancer prevention study. *Cancer Prev Res (Phila)*. 2011; 4(11):1912–1919. [PubMed: 21859925]
15. Kroemer G, Reed JC. Mitochondrial control of cell death. *Nat Med*. 2000; 6(5):513–519. [PubMed: 10802706]
16. Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, Vandenabeele P. Toxic proteins released from mitochondria in cell death. *Oncogene*. 2004; 23(16):2861–2874. [PubMed: 15077149]
17. Green DR. Apoptotic pathways: ten minutes to dead. *Cell*. 2005; 121(5):671–674. [PubMed: 15935754]
18. Basso D, Navaglia F, Fogar P, et al. DNA repair pathways and mitochondrial DNA mutations in gastrointestinal carcinogenesis. *Clinica chimica acta; international journal of clinical chemistry*. 2007; 381(1):50–55.
19. Lee CF, Liu CY, Hsieh RH, Wei YH. Oxidative stress-induced depolymerization of microtubules and alteration of mitochondrial mass in human cells. *Ann N Y Acad Sci*. 2005; 1042:246–254. [PubMed: 15965069]
20. Lee HC, Yin PH, Lu CY, Chi CW, Wei YH. Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochem J*. 2000; 348(Pt 2):425–432. [PubMed: 10816438]
21. Penta JS, Johnson FM, Wachsmann JT, Copeland WC. Mitochondrial DNA in human malignancy. *Mutation research*. 2001; 488(2):119–133. [PubMed: 11344040]
22. Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. *Clinica chimica acta; international journal of clinical chemistry*. 2004; 339(1–2):1–9.
23. Sampson MJ, Astley S, Richardson T, et al. Increased DNA oxidative susceptibility without increased plasma LDL oxidizability in Type II diabetes: effects of alpha-tocopherol supplementation. *Clin Sci (Lond)*. 2001; 101(3):235–241. [PubMed: 11524040]
24. Castro MR, Suarez E, Kraiselburd E, et al. Aging increases mitochondrial DNA damage and oxidative stress in liver of rhesus monkeys. *Exp Gerontol*. 2012; 47(1):29–37. [PubMed: 22027539]
25. SAS® software Version [9.2] of the SAS System for Unix Version [9.2]. Cary, NC, USA: SAS Institute Inc; Copyright © [9.2]
26. Acevedo-Torres K, Fonseca-Williams S, Ayala-Torres S, Torres-Ramos CA. Requirement of the *Saccharomyces cerevisiae* APN1 gene for the repair of mitochondrial DNA alkylation damage. *Environ Mol Mutagen*. 2009; 50(4):317–327. [PubMed: 19197988]
27. Bess AS, Crocker TL, Ryde IT, Meyer JN. Mitochondrial dynamics and autophagy aid in removal of persistent mitochondrial DNA damage in *Caenorhabditis elegans*. *Nucleic Acids Res*. 2012; 40(16):7916–7931. [PubMed: 22718972]
28. Siddiqui A, Rivera-Sanchez S, Castro Mdel R, et al. Mitochondrial DNA damage is associated with reduced mitochondrial bioenergetics in Huntington's disease. *Free Radic Biol Med*. 2012; 53(7):1478–1488. [PubMed: 22709585]
29. Furda AM, Marrangoni AM, Lokshin A, Van Houten B. Oxidants and not alkylating agents induce rapid mtDNA loss and mitochondrial dysfunction. *DNA Repair (Amst)*. 2012; 11(8):684–692. [PubMed: 22766155]
30. Redon CE, Dickey JS, Nakamura AJ, et al. Tumors induce complex DNA damage in distant proliferative tissues in vivo. *PNAS*. 2010; 107(42):17992–17997. [PubMed: 20855610]
31. Seager AL, Shah U-K, Mikhail JM, et al. Pro-oxidant Induced DNA Damage in Human Lymphoblastoid Cells: Homeostatic Mechanisms of Genotoxic Tolerance. *Toxicol Sci*. 2012; 128(2):387–397. [PubMed: 22539617]
32. Santos JH, Hunakova Lu, Chen Y, Bortner C, Van Houten B. Cell Sorting Experiments Link Persistent Mitochondrial DNA Damage with Loss of Mitochondrial Membrane Potential and Apoptotic Cell Death. *J Biol Chem*. 2003; 278(3):1728–1734. [PubMed: 12424245]
33. Ruchko M, Gorodnya O, LeDoux SP, Alexeyev MF, Al-Mehdi A-B, Gillespie MN. Mitochondrial DNA damage triggers mitochondrial dysfunction and apoptosis in oxidant-challenged lung

- endothelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2005; 288(3):L530–L535. [PubMed: 15563690]
34. Liu CS, Tsai CS, Kuo CL, et al. Oxidative stress-related alteration of the copy number of mitochondrial DNA in human leukocytes. *Free Radic Res*. 2003; 37(12):1307–1317. [PubMed: 14753755]
 35. Shen J, Platek M, Mahasneh A, Ambrosone C, Zhao H. Mitochondrial copy number and risk of breast cancer: a pilot study. *Mitochondrion*. 2010; 10(1):62–68. [PubMed: 19788937]
 36. Lan Q, Lim U, Liu CS, et al. A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. *Blood*. 2008; 112(10):4247–4249. [PubMed: 18711000]
 37. Xing J, Chen M, Wood CG, et al. Mitochondrial DNA content: its genetic heritability and association with renal cell carcinoma. *Journal of the National Cancer Institute*. 2008; 100(15): 1104–1112. [PubMed: 18664653]
 38. Yu M, Zhou Y, Shi Y, et al. Reduced mitochondrial DNA copy number is correlated with tumor progression and prognosis in Chinese breast cancer patients. *IUBMB Life*. 2007; 59(7):450–457. [PubMed: 17654121]
 39. Meierhofer D, Mayr JA, Foetschl U, et al. Decrease of mitochondrial DNA content and energy metabolism in renal cell carcinoma. *Carcinogenesis*. 2004; 25(6):1005–1010. [PubMed: 14764459]
 40. Radpour R, Fan AX, Kohler C, Holzgreve W, Zhong XY. Current understanding of mitochondrial DNA in breast cancer. *Breast J*. 2009; 15(5):505–509. [PubMed: 19624415]
 41. Basso D, Greco E, Fogar P, et al. Pancreatic cancer-derived S-100A8 N-terminal peptide: a diabetes cause? *Clinica chimica acta; international journal of clinical chemistry*. 2006; 372(1–2): 120–128.
 42. Tyrberg B, Anachkov KA, Dib SA, Wang-Rodriguez J, Yoon KH, Levine F. Islet expression of the DNA repair enzyme 8-oxoguanosine DNA glycosylase (Ogg1) in human type 2 diabetes. *BMC endocrine disorders*. 2002; 2(1):2. [PubMed: 12003641]
 43. Koorstra JB, Hong SM, Shi C, et al. Widespread activation of the DNA damage response in human pancreatic intraepithelial neoplasia. *Mod Pathol*. 2009; 22(11):1439–1445. [PubMed: 19668150]
 44. Collins AR, Oscoz AA, Brunborg G, et al. The comet assay: topical issues. *Mutagenesis*. 2008; 23(3):143–151. [PubMed: 18283046]
 45. Matta JL, Villa JL, Ramos JM, et al. DNA repair and nonmelanoma skin cancer in Puerto Rican populations. *J Am Acad Dermatol*. 2003; 49(3):433–439. [PubMed: 12963906]
 46. Sturgis EM, Clayman GL, Guan Y, Guo Z, Wei Q. DNA repair in lymphoblastoid cell lines from patients with head and neck cancer. *Arch Otolaryngol Head Neck Surg*. 1999; 125(2):185–190. [PubMed: 10037285]
 47. Orlov I, Park BJ, Mujumdar U, et al. DNA damage and repair capacity in patients with lung cancer: prediction of multiple primary tumors. *J Clin Oncol*. 2008; 26(21):3560–3566. [PubMed: 18640936]
 48. Kennedy DO, Agrawal M, Shen J, et al. DNA repair capacity of lymphoblastoid cell lines from sisters discordant for breast cancer. *Journal of the National Cancer Institute*. 2005; 97(2):127–132. [PubMed: 15657342]
 49. Matta J, Echenique M, Negron E, et al. The association of DNA Repair with breast cancer risk in women. A comparative observational study. *BMC Cancer*. 2012; 12:490. [PubMed: 23088658]
 50. Perez-Mayoral J, Pacheco-Torres AL, Morales L, Acosta-Rodriguez H, Matta JL, Dutil J. Genetic polymorphisms in RAD23B and XPC modulate DNA repair capacity and breast cancer risk in Puerto Rican women. *Mol Carcinog*. 2013
 51. Ramos JM, Ruiz A, Colen R, Lopez ID, Grossman L, Matta JL. DNA repair and breast carcinoma susceptibility in women. *Cancer*. 2004; 100(7):1352–1357. [PubMed: 15042667]
 52. Santos RA, Teixeira AC, Mayorano MB, Carrara HH, Andrade JM, Takahashi CS. Basal levels of DNA damage detected by micronuclei and comet assays in untreated breast cancer patients and healthy women. *Clin Exp Med*. 2010; 10(2):87–92. [PubMed: 19902326]
 53. Hunter SE, Jung D, Di Giulio RT, Meyer JN. The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. *Methods*. 2010; 51(4):444–451. [PubMed: 20123023]

54. Meyer JN. QPCR: a tool for analysis of mitochondrial and nuclear DNA damage in ecotoxicology. *Ecotoxicology*. 2010; 19(4):804–811. [PubMed: 20049526]

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Table 1

Characteristics of pancreatic adenocarcinoma cases and controls.

	Cases (N=48)	Controls (N=48)
Sex		
Female	25 (52.1%)	21 (43.8%)
Male	23 (47.9%)	27 (56.2%)
Age when approached		
Mean (SD)	65.4 (2.94)	65.3 (2.50)
Median	66.0	65.0
Q1, Q3	63.0, 68.0	64.0, 67.0
Range	(60.0–69.0)	(60.0–69.0)
Diabetes Mellitus Type 2		
Yes	18 (37.5%)	8 (16.7%)
Onset 3 years ago	35 (9.1%)	43 (4.4%)
Onset < 3 years ago	120 (31.3%)	21 (2.1%)
No	30 (62.5%)	40 (83.3%)
Smoking		
Never	48 (100%)	48 (100%)
Usual BMI		
Mean (SD)	28.4 (5.66)	27.7 (4.81)
Median	27.2	27.0
Q1, Q3	24.2, 31.1	23.7, 31.3
Range	(19.0–45.5)	(19.1–38.7)
Alcohol (drinks/week)		
Mean (SD)	0.8 (1.46)	0.8 (1.20)
Median	0.3	0.3
Q1, Q3	0.1, 1.0	0.1, 1.0
Range	(0.0–11.3)	(0.0–11.3)

Abbreviations : SD, standard deviation; Q1, 25th percentile; Q3, 75th percentile; Range (minimum-maximum)

Quantitative PCR measured amplification for mitochondrial DNA (mtDNA), nuclear DNA (nDNA), and mitochondrial abundance from peripheral blood mononuclear cells in pancreatic adenocarcinoma cases and controls. Higher amplification reflects lower DNA damage.

Table 2

Mean (95% CI)		Median (range)	rank sum p-value	Unadjusted			Adjusted ^a		
				OR (95% CI)	p-value		OR (95% CI)	p-value	
Average mtDNA Amplification^{*bc}									
Control	355.33 (332.21 , 378.46)	359.85 (191.6–605.87)	0.03	1.004 (0.998, 1.009)	0.18	1.004 (0.998, 1.009)	1.004 (0.998, 1.009)	0.17	
Case	378.04 (353.51 , 402.56)	395.13 (90.25–541.66)							
Average mtDNA Abundance^{*b}									
Control	178.81 (168.2, 189.42)	170.69 (116.44–307.8)	0.62	0.997 (0.987, 1.008)	0.59	1 (0.989, 1.011)		0.95	
Case	174.51 (162.27 , 186.74)	173.44 (70.61–311.27)							
Average Relative nDNA Amplification^{*b}									
Control	1.06 (0.94 , 1.18)	1.09 (0.16–1.88)	0.08	2.1 (0.791, 5.572)	0.14	2.561 (0.88, 7.456)		0.08	
Case	1.21 (1.05 , 1.37)	1.24 (0.04–2.37)							

* square root transformation was used to normalize distribution
^a Logistic regression adjusted for age, sex, and type 2 diabetes
^b Corrected for no DNA amplification reaction controls
^c Normalized to mtDNA abundance

Table 3

Quantitative PCR measured amplification for mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) from peripheral blood mononuclear cells based on reported type 2 diabetes mellitus (T2DM) status.

	Mean (95% CI)	Median (range)	rank sum p-value
Average mtDNA Amplification^{*ab}			
DM-No	366.37 (348.78 , 383.96)	368.76 (199.38–605.87)	0.78
DM-Yes	366.66 (324.31 , 409.01)	389.08 (90.25–562.81)	
Average mtDNA Abundance^{*a}			
DM-No	180.98 (172.2 , 189.76)	175.44 (116.44–307.8)	0.04
DM-Yes	165.02 (147.28 , 182.76)	160.59 (70.61–311.27)	
Average Relative nDNA Amplification^{*a}			
DM-No	1.15 (1.05 , 1.24)	1.17 (0.19–1.92)	0.90
DM-Yes	1.1 (0.82 , 1.38)	1.21 (0.04–2.37)	

Number of self-reported non-diabetics for cases = 30 and for controls = 40; number of self-reported diabetics for cases = 18 and for controls = 8.

^{*} square root transformation was used to normalize distribution

^a Corrected for no DNA amplification reaction in controls

^b Normalized to mtDNA abundance