# Mitochondrial Genetic Polymorphisms and Pancreatic Cancer Risk

**Liang Wang,[1] William R. Bamlet,[2] Mariza de Andrade,[2] Lisa A. Boardman,[3] Julie M. Cunningham,[1] Stephen N. Thibodeau,[1] and Gloria M. Petersen[2]**

Departments of [1]Laboratory Medicine and Pathology, [2]Health Sciences Research, and [3]Gastroenterology, Mayo Clinic College of Medicine, Rochester, Minnesota

## Abstract

**The role of genes that influence the risk of developing pancreatic cancer (PC) has not been well studied. The mitochondrion, conventionally thought to be an organelle specific to energy metabolism, is in fact multifunctional and has been implicated in many diseases, including cancer. To evaluate whether single nucleotide polymorphisms in mitochondrial DNA (mtSNP) are associated with increased risk of PC, we screened Caucasian cases diagnosed or seen at the Mayo Clinic with primary pancreatic adenocarcinoma ($n$ = 955), and healthy clinic-based Caucasian controls ($n$ = 1,102). A total of 24 mtSNPs, including 10 of the most common tagSNPs, 7 non-tagSNPs in the coding region, and 7 common SNPs in the regulatory region were genotyped. For analysis, these samples were grouped into two phases, the ''testing'' set (474 cases and 615 controls), and the ''validation'' set (481 cases and 487 controls). In the testing set, one mtSNP (SNP11719) suggested an association in single SNP analysis, with an odds ratio of 1.34 (95% confidence intervals, 1.05-1.72; $P$ = 0.020), but did not remain statistically significant after correction for multiple testing. In the validation set, none of the 24 variants indicated any association with PC. For haplogroup analysis, 10 core SNPs that form common haplogroups in Caucasians (1719, 4580, 7028, 8251, 9055, 10398, 12308, 13368, 13708, and 16391) were evaluated. No significant associations with PC were identified either by analyzing the two sets separately or combined (combined global $P$ = 0.17). Overall, these results do not support a significant involvement of mitochondrial DNA variation in the risk of developing PC. Investigation of other mitochondrial genetic variations (i.e., nuclear-encoded mitochondrial proteins) would be necessary to elucidate any role of mitochondrial DNA variation in PC.** (Cancer Epidemiol Biomarkers Prev 2007;16(7):1455–9)

## Introduction

Pancreatic cancer (PC) will affect ~37,170 individuals in the U.S. in 2007, and lead to 33,370 deaths, making PC the fourth leading cause of cancer death in the U.S. (1). Five-year survival rates for patients suffering from this cancer are a dismal 5%, emphasizing the need for a detailed understanding of the molecular changes underlying this disease and the application of this knowledge to early diagnosis and therapeutic intervention. Several etiologic factors for PC have been suggested, including both genetic and environmental factors. These risk factors include age, smoking, diabetes, gender, race/ethnicity, family history, and chronic and hereditary pancreatitis. PC is rare before the age of 40 years, but incidence increases sharply with increasing age; most patients are diagnosed between the ages of 60 and 80.

The mitochondrion, an organelle specific to energy metabolism, is in fact multifunctional, having roles in cell signaling, apoptosis, and cellular homeostasis. Mitochondria can activate apoptosis, during which reactive oxygen species are generated that on one hand serve as crucial proapoptotic factors, but on the other hand, function in both the initiation and promotion of carcinogenesis. Mitochondrial dysfunction is a common feature of cancer cells. Somatic mutations of mitochondrial DNA have been reported in a variety of cancers, including PC (2-9). Intragenic deletions (10), missense and chain-terminating point mutations (3), and alterations of homopolymeric sequences (11) have been identified in nearly every type of tumor studied.

Recently, several reports have shown that mitochondrial enzymes are directly implicated in hereditary cancer syndromes (12). For example, complex II of the mitochondrial respiratory chain is composed of four nuclear-encoded subunits and is localized in the mitochondrial inner membrane. Germ line heterozygous mutations in three of the four subunits (SDHB, SDHC, and SDHD) cause the inherited syndromes that feature phaeochromocytoma and paraganglioma (13). Another example is the nuclear-encoded mitochondrial enzyme, fumarase (*FH*). The protein is an enzymatic component of the tricarboxylic acid cycle and catalyzes the formation of L-malate from fumarate. Mutations in the *FH* gene cause a predisposition to cutaneous and uterine leiomyomas, as well as to kidney cancers (14). More recent studies showed that single nucleotide polymorphisms in the mitochondrial genome (mtSNP) were associated with increased risk of several types of cancers, including invasive breast cancer in African American women (15), and poor outcome of PC (6). These findings provide evidence that DNA variation in mitochondria may play an important role in developing PC, a hypothesis recently emphasized in a conference report (16). To systematically test the role of mtSNPs in PC risk, we genotyped 24 common variants, including 10 of the most common tagSNPs and 7 non-tagSNPs in the coding region, as well as 7 common SNPs in the regulatory region. We compared allele and haplogroup frequencies in a total of 955 cases with primary pancreatic adenocarcinoma and 1,102 healthy clinic-based controls.

## Materials and Methods

**Patients with PC.** PC index cases were adult persons with a histologically confirmed primary adenocarcinoma of the pancreas seen at Mayo Clinic between October 1, 2000 and

Received 2/7/07; revised 4/28/07; accepted 5/10/07.

**Grant support:** National Cancer Institute P50 CA102701 (Mayo Clinic Specialized Programs of Research Excellence in Pancreatic Cancer).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Liang Wang, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, 200 First Street Southwest, Stabile 241, Rochester, MN 55905. Phone: 507-284-9136; Fax: 507-266-5193. E-mail: wang.liang@mayo.edu

Copyright © 2007 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-07-0119



Downloaded from cebp.aacrjournals.org on April 22, 2019. © 2007 American Association for Cancer Research.

**Table 1. Characteristics of PC cases and controls**

| | Testing set | | | Validation set | | |
|---|---|---|---|---|---|---|
| | Control ($N$ = 615) | PC case ($N$ = 474) | $P$ | Control ($N$ = 487) | PC case ($N$ = 481) | $P$ |
| Age | 59.8 ± 12.2 | 65.8 ± 10.4 | <0.001 | 65.3 ± 10.5 | 66.3 ± 10.9 | 0.16 |
| Gender | | | 0.018 | | | 0.50 |
| Female | 292 (47%) | 191 (40%) | | 217 (45%) | 204 (42%) | |
| Male | 323 (53%) | 283 (60%) | | 270 (55%) | 277 (58%) | |
| Smoking history | | | 0.011 | | | <0.001 |
| Never smoker | 297 (49%) | 193 (41%) | | 216 (50%) | 167 (35%) | |
| Smoker | 315 (51%) | 280 (59%) | | 216 (50%) | 304 (65%) | |
| Questionnaire completed | | | | | | |
| No | | | | 50 (10%) | 149 (31%) | |
| Yes | | | | 437 (90%) | 332 (69%) | |
| Body mass index | | 28.2 ± 5.6 | | 27.2 ± 4.6 | 27.9 ± 5.0 | 0.06 |
| Diabetes mellitus (>2 y) | | | | | | <0.001 |
| No | | 329 (90%) | | 418 (96%) | 287 (86%) | |
| Yes | | 35 (10%) | | 17 (4%) | 45 (14%) | |

June 1, 2006. Eligible Mayo pancreatic adenocarcinoma cases were identified through an ultra-rapid patient identification system. Study coordinators identified potential patients from the electronic patient schedule system and daily pathology reports. All eligible patients were contacted either in the clinic at the time of their appointment, or later by mail or phone if clinic contact was not possible. If contacted at the clinic, a research nurse obtained informed consent, arranged a venipuncture for 40 mL of blood, and asked the participant to complete the study questionnaire. If mail contact was required (~28% of the cases were approached by mail), the study coordinator mailed an invitation letter to the patient's home address. A follow-up telephone call was made if the sample or forms were not received after 1 month. Approximately 74% of all eligible patients were enrolled in this study. The characteristics of the Caucasian subjects used in this study are listed in Table 1. We included 474 patients in the testing set and 481 patients in the validation set.

**Controls.** The 615 healthy Caucasian controls in the testing set were individuals who were identified through a colon cancer screening study between June 1, 2000 and May 31, 2004. These subjects were recruited after completion of a routine screening colonoscopy which was negative for colon cancer. This control group was a convenience sample and therefore was not selected to match the pancreas cancer cases. Self-reported height, weight, and diabetes status were not available on these individuals. The 487 healthy Caucasian controls in the validation set were from a Mayo Clinic–based control sample of primary care patients having routine check-up visits (general medical exam) between May 1, 2004 and August 31, 2006. Controls were frequency-matched on age (±5 years), race, sex, and state/region of residence distribution of the cases. Controls had no previous diagnosis of cancer (except non–melanoma skin cancer) at the time of enrollment. Prior to their appointment, potential controls were mailed an information brochure describing the study and a letter of invitation. On the day of the appointment, a study assistant approached the subject, confirmed eligibility criteria, and obtained informed consent. Each participant completed study questionnaires (which included a self-report of height, weight, and diabetes status) and provided 30 mL of research blood sample. Approximately 70% of all approached controls participated in this study.

**SNP Selection.** To date, thousands of mtSNPs have been reported.[4] However, the majority are rare (allele frequency < 1%). Based on allele frequency and published literature (17), we selected a total of 24 SNPs distributed across the mitochondrial genome for genotyping (Table 2). Ten of the 24 SNPs define common haplogroups in the Caucasian population (18). For the mitochondrial coding region, Saxena et al. (17) identified 144 SNPs with minor allele frequencies ≥ 1% and 64 tagSNPs to capture them with $r^2 \geq 0.8$. By considering sample sizes, we selected the top 10 most common tagSNPs and 7 non-tagSNPs (necessary for haplogroup analysis) in the coding region. Because a non-tagSNP may have high correlation with multiple tagSNPs (e.g., non-tagSNP1719 is correlated with tagSNPs 709, 12007, and 12705 with $r^2 = 0.84$), the 7 non-tagSNPs actually capture 13 extra tagSNPs with $r^2 > 0.8$. Altogether, our 17 SNPs in the coding region captured 23 of 64 tagSNPs with minor allele frequencies ≥ 1%, 10 of 15 tagSNPs with minor allele frequencies ≥ 5%, and 10 of 10 tagSNPs with minor allele frequencies ≥ 7%. For the regulatory region (displacement loop or D-loop), we selected 7 SNPs based on higher allele frequency or necessity for haplogroup analysis.

**SNP Genotyping.** We used the Beckman SNPstream system for genotyping. Two 12 plex panels of primer sets were designed using the Web-based Autoprimer. For each 12 plex panel, 2 ng of DNA isolated from peripheral blood lymphocytes was amplified with the pooled primer sets (50 nmol/L each) under universal PCR conditions (5 mmol/L $MgCl_2$, 75 μmol/L of deoxynucleotide triphosphates, 0.1 unit of AmpliTaq Gold; Applied Biosystems) in a final volume of 5 μL. After initial denaturation at 94°C for 1 min, 34 cycles were done at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The amplified materials were then cleaned by incubating with SBE Clean-up (shrimp alkaline phosphatase and exonuclease I) at 37°C for 30 min and 96°C for 10 min. After 9 μL of extension mixes for each panel (C/T or G/A) were added to 7 μL of cleaned PCR product, the plates were thermocycled at 96°C for 3 min, then 45 cycles of 94°C for 20 s and 40°C for 11 s. SNPware array plates were prepared (washing with buffers I and II). Eight microliters of hybridization solution was added to each well of the plate following primer extension reaction, and 10 μL of this was added to the corresponding well in the SNPware tag plate, incubated at 42°C at 100% humidity for 2 h. The tag arrays were then washed and vacuum-dried and imaged on the scanner. The SNPstream software was used for image data analysis and genotype calls.

**Single SNP Analysis.** Although there are multiple mitochondrial DNA copies per cell, there is generally only a single allele for any given individual (maternal inheritance). The mtSNP allele frequency was used to assess the difference between the cases and controls using standard contingency

[4] http://www.mitomap.org


Downloaded from cebp.aacrjournals.org on April 22, 2019. © 2007 American Association for Cancer Research.

**Table 2. Analysis of 24 mtSNPs in patients with PC and controls**

| Position and variant | Location | Accession no., if known | Testing set | | | Validation set | | | Combined set | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control (*N* = 615) | PC case (*N* = 474) | *P** | Control (*N* = 487) | PC case (*N* = 481) | *P** | Control (*N* = 1,102) | PC case (*N* = 955) | *P** |
| 73G/A | D-loop | – | 338/248 | 255/212 | 0.32 | 258/222 | 248/229 | 0.59 | 596/470 | 503/441 | 0.24 |
| 152T/C | D-loop | – | 427/102 | 358/95 | 0.51 | 364/112 | 358/105 | 0.76 | 791/214 | 716/200 | 0.77 |
| 489C/T | D-loop | – | 59/519 | 52/407 | 0.56 | 43/424 | 48/412 | 0.53 | 102/943 | 100/819 | 0.41 |
| 709A/G | 12S rRNA | – | 88/483 | 21/126 | 0.73 | 68/417 | 61/417 | 0.57 | 156/900 | 825/43 | 0.35 |
| 1189C/T | 12S rRNA | – | 44/541 | 33/431 | 0.80 | 32/455 | 35/441 | 0.63 | 76/996 | 68/872 | 0.90 |
| 1719A/G | 16S rRNA | – | 34/560 | 28/442 | 0.87 | 26/460 | 15/465 | 0.09 | 60/1,020 | 43/907 | 0.29 |
| 3010G/A | 16S rRNA | – | 401/119 | 321/127 | 0.05 | 365/121 | 363/114 | 0.72 | 766/240 | 684/241 | 0.26 |
| 4580A/G | ND2 | – | 24/558 | 19/451 | 0.95 | 18/467 | 20/460 | 0.72 | 42/1,025 | 39/911 | 0.85 |
| 7028T/C | COI | rs2015062 | 356/223 | 265/203 | 0.11 | 275/210 | 271/206 | 0.97 | 631/433 | 536/409 | 0.24 |
| 8251A/G | COII | rs3021089 | 31/567 | 21/450 | 0.58 | 26/455 | 21/438 | 0.56 | 57/1,022 | 42/888 | 0.43 |
| 9055G/A | ATPase 6 | – | 535/56 | 426/43 | 0.86 | 438/48 | 430/47 | 0.99 | 973/104 | 856/90 | 0.91 |
| 10398G/A | ND3 | rs2853826 | 118/464 | 95/365 | 0.88 | 98/385 | 97/374 | 0.91 | 216/849 | 192/739 | 0.85 |
| 11719A/G | ND4 | rs2853495 | 322/239 | 234/233 | 0.02 | 248/232 | 231/244 | 0.35 | 570/471 | 465/477 | 0.016 |
| 11812G/A | ND4 | rs16822406 | 46/539 | 33/433 | 0.63 | 33/453 | 21/456 | 0.11 | 79/992 | 54/889 | 0.14 |
| 12308G/A | tRNA | rs2853498 | 148/437 | 99/365 | 0.13 | 109/376 | 109/369 | 0.90 | 257/813 | 208/734 | 0.30 |
| 12372A/G | ND5 | rs2853499 | 151/429 | 103/368 | 0.12 | 112/372 | 111/369 | 1.00 | 263/801 | 214/737 | 0.24 |
| 12705T/C | ND5 | rs2854122 | 47/541 | 37/432 | 0.95 | 35/451 | 34/444 | 0.96 | 82/992 | 71/876 | 0.91 |
| 13368G/A | ND5 | rs3899498 | 526/58 | 424/44 | 0.77 | 440/47 | 439/38 | 0.36 | 966/105 | 863/82 | 0.38 |
| 13708A/G | ND5 | – | 71/497 | 59/409 | 0.96 | 69/418 | 56/422 | 0.26 | 140/915 | 115/831 | 0.46 |
| 14798T/C | Cytb | – | 484/100 | 399/69 | 0.30 | 406/78 | 400/80 | 0.82 | 890/178 | 799/149 | 0.56 |
| 16126C/T | D-loop | – | 119/427 | 91/375 | 0.38 | 101/385 | 85/393 | 0.24 | 220/812 | 176/768 | 0.14 |
| 16189T/C | D-loop | – | 476/86 | 395/73 | 0.90 | 419/63 | 419/56 | 0.55 | 895/149 | 814/129 | 0.70 |
| 16391G/A | D-loop | – | 572/16 | 458/11 | 0.70 | 473/13 | 467/10 | 0.56 | 104/529 | 925/21 | 0.49 |
| 16519C/T | D-loop | – | 367/204 | 303/159 | 0.66 | 307/180 | 296/180 | 0.78 | 674/384 | 599/339 | 0.94 |

*$P$ values were estimated prior to statistical adjustment for covariates (age, gender, and smoking history).

table methods. Unconditional logistic regression models, which treated case/control status as the outcome, were used to test the association between PC risk and SNP carrier status adjusting for covariates (age, gender, and smoking history). Odds ratios (OR) and 95% confidence intervals (95% CI) were used to quantify any significant associations. Similar analyses were done on the validation set. Additional analyses which included body mass index and history of diabetes exceeding 2 years as covariates were also investigated in the validation set. To account for multiple testing, Bonferroni correction was used. Analyses were done using SAS version 9.1.3.

**Haplogroup Analysis.** Genotypes for the multiple mtSNPs were combined to construct mitochondrial haplogroups. Haplogroups are specific combinations of nucleotides on the same mitochondrial genomes. To test for an association between the haplogroup and case/control status, we calculated a score statistic using a modified version of the haplo.stats program (19) which implements an expectation-maximization algorithm to infer missing haplogroups. Analyses using the haplo.stats software were run using S-plus version 7.0.6. We then obtained estimates of ORs and 95% CIs for haplogroups using unconditional logistic regression models in SAS version 9.1.3.

## Results

We genotyped 24 SNPs distributed across the mitochondrial genome. We first examined quality control samples to assess the reliability of genotyping. There were 44 positive (same anonymous DNA) and 44 negative (no DNA) controls in the entire data set. There were also control spots embedded within each of the wells of the tag array plate for each homozygous allele, one heterozygous and one negative control. Final analysis revealed no sign of contamination or other technical problems associated with the genotype call. Except for mtSNP709, which failed quality control in some cases in the testing set, the overall median call rate for each of the groups ranged from 94.6% to 99.6%. The frequencies of the mtSNPs are shown in Table 2, organized by the testing set, the validation set, and the combined set. In our initial unadjusted analysis, we did not detect any significant association between cases and controls in 23 of the 24 mtSNPs. The only SNP that

**Table 3. Analysis of 10 haplogroups in PC cases and controls**

| Haplogroup | Testing set | | | Validation set | | | Combined set | | |
|---|---|---|---|---|---|---|---|---|---|
| | Controls (*N* = 615) | Cases (*N* = 474) | OR* (95% CI) | Controls (*N* = 487) | Cases (*N* = 481) | OR* (95% CI) | Controls (*N* = 1,102) | Cases (*N* = 955) | OR* (95% CI) |
| H | 210 | 197 | 1 (–) | 206 | 191 | 1 (–) | 416 | 388 | 1 (–) |
| I | 12 | 10 | 0.888 (0.375-2.102) | 13 | 9 | 0.747 (0.312-1.787) | 25 | 19 | 0.815 (0.442-1.503) |
| J | 51 | 40 | 0.836 (0.529-1.321) | 49 | 43 | 0.946 (0.601-1.491) | 100 | 83 | 0.890 (0.645-1.228) |
| K | 40 | 31 | 0.826 (0.497-1.373) | 31 | 33 | 1.148 (0.677-1.947) | 71 | 64 | 0.966 (0.671-1.392) |
| T | 54 | 39 | 0.770 (0.488-1.214) | 44 | 416 | 0.858 (0.528-1.394) | 98 | 74 | 0.810 (0.581-1.128) |
| U | 95 | 64 | 0.718 (0.495-1.042) | 77 | 70 | 0.980 (0.671-1.432) | 172 | 134 | 0.835 (0.641-1.089) |
| V | 23 | 18 | 0.834 (0.437-1.593) | 18 | 20 | 1.198 (0.615-2.334) | 41 | 38 | 0.994 (0.626-1.578) |
| W | 8 | 5 | 0.666 (0.214-2.071) | 9 | 7 | 0.839 (0.306-2.297) | 17 | 12 | 0.757 (0.357-1.605) |
| X | 10 | 8 | 0.853 (0.330-2.205) | 6 | 2 | 0.360 (0.072-1.803) | 16 | 10 | 0.670 (0.300-1.494) |
| Com † | 22 | 24 | 1.163 (0.632-2.141) | 16 | 32 | 2.157 (1.147-4.057) | 38 | 56 | 1.580 (1.023-2.440) |

*ORs were estimated prior to statistical adjustment for covariates (age, gender, and smoking history).
†A composite group of two less frequent haplogroups.


Downloaded from cebp.aacrjournals.org on April 22, 2019. © 2007 American Association for Cancer Research.

showed an association was mtSNP11719 (accession no. rs2853495). The minor allele frequencies (C allele) for this SNP in the testing set was 50% in PC and 43% in controls. The OR was 1.34 with 95% CI between 1.05 and 1.72 ($P = 0.02$). However, mtSNP11719 did not show an association with PC in the validation set (OR, 1.13; 95% CI, 0.87-1.46). After adjustment for age, gender, and smoking history, two additional mtSNPs (mtSNP3010 and mtSNP1719) showed an association with PC. The association for mtSNP3010 (OR, 1.45; 95% CI, 1.07-1.96) was observed in the testing set and the association for mtSNP1719 (OR, 2.03; 95% CI, 1.02-4.03) was observed in the validation set. Additional analyses which also adjusted for body mass index and history of diabetes exceeding 2 years were done in the validation set and provided consistent results (data not shown).

Rather than just leaving persons with missing data out of the analysis, we also conducted a sensitivity analysis to determine the effect of missing information on covariates. Because the questionnaires were available for all cases and only for the controls in the validation set, we did our sensitivity analysis using only the validation set. We evaluated minimally adjusted ORs, and then adjusted for the additional covariates on the reduced data set. Similar results were observed when persons were removed with missing information.

To analyze mitochondrial haplogroups, we used 10 core mtSNPs that defined common haplogroups in Caucasians (18): SNPs 1719, 4580, 7028, 8251, 9055, 10398, 12308, 13368, 13708, and 16391. Haplogroups were constructed using an expectation-maximization algorithm to allow for missing data. Eleven haplogroups with frequencies of at least 1% were included in the analysis. The analysis was based on global score statistics that compare all 11 haplogroups between cases and controls. This approach, which controls for multiple testing, did not identify a significant association with PC (combined global $P = 0.17$). Two haplogroups that occurred less frequently (0.011 and 0.042) seemed to be driving the global $P$ value in the combined analysis. These two less frequent haplogroups were combined into a composite (Com) group. The final analyses were done in 10 haplogroups (Table 3). When haplogroups were examined individually, however, haplogroup Com showed a positive association with PC in the validation set only, and its frequency was 0.07 in cases and 0.03 in controls (OR, 2.16; 95% CI, 1.15-4.06). The combined set did show a similar pattern for association of the haplogroup Com with PC risk (Table 3). This association also remained significant in the validation set after adjusting for age, gender, and smoking history (OR, 2.26; 95% CI, 1.15-4.42). However, the association did not remain significant in the combined set after adjusting for age, gender, and smoking history.

## Discussion

In this study, we genotyped and compared 24 mtSNPs and 11 common haplogroups in Caucasian PC cases and clinic-based healthy controls. Although the overall statistical analysis did not reveal significant differences for these mtSNPs/haplogroups between controls and PC cases, some individual SNP or haplogroup combinations did show weak associations. The mtSNP11719 was the only SNP that showed an association before statistical adjustment, and is located in the coding region of the ND4 gene. As this variant does not cause an amino acid change (synonymous change), it is unlikely to be causal if it indeed is associated with PC risk. However, the recent report of altered *in vivo* protein folding for a synonymous *MRD1* variant (20) does indicate that synonymous variants should not *a priori* be assumed to be nonfunctional. After adjustment for covariates (age, gender, and smoking history), two additional mtSNPs (3010 and 1719) showed associations with PC. The mtSNP3010 is located at the coding region of 16S rRNA and the mtSNP1719 is also located at the coding region of 16S rRNA. Therefore, both may potentially cause functional changes. However, these associations were observed in only one of our two data sets, suggesting that these associations should be interpreted carefully and may require further evaluation.

Navaglia et al. recently tested four mtSNPs (73, 152, 16189, and 16519) in the D-loop region for association with PC risk using 99 cases with PC and 87 controls, and found that none were correlated with the disease (6). Our current study strengthens their observation that these mtSNPs do not predispose to PC (Table 2). However, Navaglia et al. also reported a significant association of the mtSNP16519 with PC-associated diabetes mellitus and prognosis (6). In our preliminary analysis of 654 non–surgery patients and 366 surgery patients (separately assessed), we observed no correlation of this mtSNP with PC survival. Clearly, detailed analysis is needed to elucidate the potential prognostic role of this SNP in PC patients.

When considering each haplogroup individually, we observed an association between haplogroup Com and PC risk in both the validation set and the combined set (Table 3). Studies have shown that inherited polymorphisms are functionally different. For example, different mitochondrial haplogroups are qualitatively different from each other. Haplogroups H and T displayed a significant difference in the activity of complexes I and IV of oxidative phosphorylation (21). Individuals selected for longevity (male centenarians) had a significantly higher frequency of the European haplogroup J than sex-matched younger subjects having the same ethnic and geographic origin (22, 23). Therefore, although our observation may be a chance association, haplogroup-specific effects on PC risk and rate of aging may merit further study.

Recent data suggest that inherited variation of mitochondrial DNA could affect the occurrence of mutations (24). The acquired mutations are not randomly distributed across the mitochondrial genome, and the rate of mutation accumulation seems to vary with different genetic background. Yeh et al. (25) have shown that the distribution and spectra of germline mitochondrial gene variants seemed to differ between individuals with thyroid cancer compared with normal controls, with complex I variants favored among cancer cases. Maximo et al. (26) reported that germline polymorphisms of the mitochondrial *ATPase 6* gene were associated with the occurrence of mitochondrial DNA common deletion in thyroid tumors. Because *ATPase 6* may have a role in mitochondrial DNA maintenance, these polymorphisms in *ATPase 6* could lead to a less efficient mitochondrial DNA replication and to mitochondrial DNA abnormalities that could contribute to the occurrence of mitochondrial DNA common deletions and tumorigenesis.

Overall, our results do not support a significant involvement of mtSNPs or haplogroups in PC risk. Much larger numbers will be needed to achieve the power required to confirm the suggestive associations we have observed, which could also be due to chance. Because this study did not represent an exhaustive investigation of mitochondrial genetic variation (especially for nuclear-encoded mitochondrial proteins), additional work would be necessary to fully elucidate the role of mitochondrial genetic variation in PC.

## References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Singh KK. Mitochondrial dysfunction is a common phenotype in aging and cancer. Ann N Y Acad Sci 2004;1019:260–4.
3. Polyak K, Li Y, Zhu H, et al. Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 1998;20:291–3.
4. Chen JZ, Gokden N, Greene GF, Mukunyadzi P, Kadlubar FF. Extensive somatic mitochondrial mutations in primary prostate cancer using laser capture microdissection. Cancer Res 2002;62:6470–4.


Downloaded from cebp.aacrjournals.org on April 22, 2019. © 2007 American Association for Cancer Research.

5. Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nat Rev Genet 2005;6:389–402.
6. Navaglia F, Basso D, Fogar P, et al. Mitochondrial DNA D-loop in pancreatic cancer: somatic mutations are epiphenomena while the germline 16519 T variant worsens metabolism and outcome. Am J Clin Pathol 2006; 126:593–601.
7. Maitra A, Cohen Y, Gillespie SE, et al. The Human MitoChip: a high-throughput sequencing microarray for mitochondrial mutation detection. Genome Res 2004;14:812–9.
8. Jones JB, Song JJ, Hempen PM, Parmigiani G, Hruban RH, Kern SE. Detection of mitochondrial DNA mutations in pancreatic cancer offers a ''mass''-ive advantage over detection of nuclear DNA mutations. Cancer Res 2001;61:1299–304.
9. Basso D, Navaglia F, Fogar P, et al. DNA repair pathways and mitochondrial DNA mutations in gastrointestinal carcinogenesis. Clin Chim Acta 2007;381:50–5.
10. Horton TM, Petros JA, Heddi A, et al. Novel mitochondrial DNA deletion found in a renal cell carcinoma. Genes Chromosomes Cancer 1996;15:95–101.
11. Habano W, Nakamura S, Sugai T. Microsatellite instability in the mitochondrial DNA of colorectal carcinomas: evidence for mismatch repair systems in mitochondrial genome. Oncogene 1998;17:1931–7.
12. Eng C, Kiuru M, Fernandez MJ, Aaltonen LA. A role for mitochondrial enzymes in inherited neoplasia and beyond. Nat Rev Cancer 2003;3:193–202.
13. Benn DE, Richardson AL, Marsh DJ, Robinson BG. Genetic testing in pheochromocytoma- and paraganglioma-associated syndromes. Ann N Y Acad Sci 2006;1073:104–11.
14. Sudarshan S, Linehan WM, Neckers L. HIF and fumarate hydratase in renal cancer. Br J Cancer 2007;96:403–7.
15. Canter JA, Kallianpur AR, Parl FF, Millikan RC. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. Cancer Res 2005;65:8028–33.
16. Verma M, Naviaux RK, Tanaka M, Kumar D, Franceschi C, Singh KK. Meeting report: mitochondrial DNA and cancer epidemiology. Cancer Res 2007;67:437–9.
17. Saxena R, de Bakker PI, Singer K, et al. Comprehensive association testing of common mitochondrial DNA variation in metabolic disease. Am J Hum Genet 2006;79:54–61.
18. van der Walt JM, Nicodemus KK, Martin ER, et al. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am J Hum Genet 2003;72:804–11.
19. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;70:425–34.
20. Kimchi-Sarfaty C, Oh JM, Kim IW, et al. A ''silent'' polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315:525–8.
21. Ruiz-Pesini E, Lapena AC, Diez-Sanchez C, et al. Human mtDNA haplogroups associated with high or reduced spermatozoa motility. Am J Hum Genet 2000;67:682–96.
22. De Benedictis G, Rose G, Carrieri G, et al. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. FASEB J 1999;13:1532–6.
23. Ross OA, McCormack R, Curran MD, et al. Mitochondrial DNA polymorphism: its role in longevity of the Irish population. Exp Gerontol 2001;36: 1161–78.
24. Tanaka M, Gong J, Zhang J, Yamada Y, Borgeld HJ, Yagi K. Mitochondrial genotype associated with longevity and its inhibitory effect on mutagenesis. Mech Ageing Dev 2000;116:65–76.
25. Yeh JJ, Lunetta KL, van Orsouw NJ, et al. Somatic mitochondrial DNA (mtDNA) mutations in papillary thyroid carcinomas and differential mtDNA sequence variants in cases with thyroid tumours. Oncogene 2000; 19:2060–6.
26. Maximo V, Soares P, Lima J, Cameselle-Teijeiro J, Sobrinho-Simoes M. Mitochondrial DNA somatic mutations (point mutations and large deletions) and mitochondrial DNA variants in human thyroid pathology: a study with emphasis on Hurthle cell tumors. Am J Pathol 2002;160:1857–65.




Downloaded from cebp.aacrjournals.org on April 22, 2019. © 2007 American Association for Cancer Research.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

# Mitochondrial Genetic Polymorphisms and Pancreatic Cancer Risk

Liang Wang, William R. Bamlet, Mariza de Andrade, et al.

*Cancer Epidemiol Biomarkers Prev* 2007;16:1455-1459.

| | |
|---|---|
| **Updated version** | Access the most recent version of this article at: http://cebp.aacrjournals.org/content/16/7/1455 |

| | |
|---|---|
| **Cited articles** | This article cites 26 articles, 6 of which you can access for free at: http://cebp.aacrjournals.org/content/16/7/1455.full#ref-list-1 |
| **Citing articles** | This article has been cited by 7 HighWire-hosted articles. Access the articles at: http://cebp.aacrjournals.org/content/16/7/1455.full#related-urls |

| | |
|---|---|
| **E-mail alerts** | Sign up to receive free email-alerts related to this article or journal. |
| **Reprints and Subscriptions** | To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org. |
| **Permissions** | To request permission to re-use all or part of this article, use this link http://cebp.aacrjournals.org/content/16/7/1455. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site. |

Downloaded from cebp.aacrjournals.org on April 22, 2019. © 2007 American Association for Cancer Research.