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Polymorphisms in mitochondrial genes and prostate cancer risk

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Abstract

The mitochondrion, conventionally thought to be an organelle specific to energy metabolism, is in fact multi-functional and implicated in many diseases, including cancer. To evaluate whether mitochondria-related genes are associated with increased risk for prostate cancer, we genotyped 24 single nucleotide polymorphisms (SNPs) within the mitochondrial genome (mtSNPs) and 376 tagSNPs localized to 78 nuclear-encoded mitochondrial genes. The tagSNPs were selected to achieve ≥80% coverage based on linkage disequilibrium. We compared allele and haplotype frequencies in ~1000 prostate cancer cases with ~500 population controls. An association with prostate cancer was not detected for any of the mtSNPs individually or for 10 mitochondrial common haplotypes when evaluated using a global score statistic. For the nuclear-encoded genes, none of the tagSNPs were significantly associated with prostate cancer after adjusting for multiple testing. Nonetheless, we evaluated unadjusted p-values by comparing our results with those from the CGEMS phase I data set. Seven tagSNPs had unadjusted p-values ≤ 0.05 in both our data and in CGEMS (two SNPs were identical and five were in strong linkage disequilibrium with CGEMS SNPs). These seven SNPs (rs17184211, rs4147684, rs4233367, rs2070902, rs3829037, rs7830235, and rs1203213) are located in genes *MTRR, NDUFA9, NDUFS2, NDUFB9* and *COX7A2*, respectively. Five of the seven SNPs were further included in the CGEMS phase II study, however, none of the findings for these were replicated. Overall, these results suggest that polymorphisms in the mitochondrial genome and those in the nuclear encoded mitochondrial genes evaluated are not substantial risk factors for prostate cancer.

Keywords

mitochondria; prostate cancer; genetic polymorphism; cancer risk

Introduction

In 2007, ~ 218,890 men in the U.S. will be diagnosed with prostate cancer (PC), and >27,000 deaths will be attributed to the disease (1). Several etiologic factors for PC have been suggested, including genetic and environmental factors. However, only age, race/ethnicity and family history are established risk factors (2). Age is the strongest known risk factor, and the incidence of PC rises more steeply with age than for any other cancer (3). There is a large variation in

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its incidence among men in different countries with the highest rates in the United States, Canada, Sweden, Australia, and France and Asian countries having the lowest rates (4). Although the causes of the variation of PC incidence are likely to be related to differences in screening methods, diet and health-related behaviors, clinical practice patterns, and environmental risk factors, there is a large body of literature that also strongly implicates a genetic etiology (5). This evidence comes from a variety of study designs, including case-control, cohort, twin, and family-based studies (6).

The mitochondrion, an organelle central to energy metabolism, also has multiple additional roles including cell signaling, apoptosis and cellular homeostasis. Mitochondria can generate reactive oxygen species and activate apoptosis. These reactive oxygen species function as crucial pro-apoptotic factors, but may also be involved in both initiation and promotion of carcinogenesis. Interestingly, mitochondrial dysfunction has been found to be a common feature of cancer cells. Somatic mutations of mitochondrial DNA have been reported in a variety of cancers, including PC (7–13). Somatic alterations include intragenic deletions (14), missense and chain-terminating point mutations (7), and alterations of homopolymeric sequences (15), and these have been identified in nearly every type of tumor studied.

In addition to somatic alterations, several reports have demonstrated that alterations in mitochondrial enzymes are also implicated in hereditary cancer syndromes (16). For example, the complex II of mitochondrial respiratory chain is composed of four nuclear-encoded subunits and is localized in the mitochondrial inner membrane. Germline heterozygous mutations in three of the four subunits (*SDHB, SDHC* and *SDHD*) cause the inherited syndromes that feature phaeochromocytoma and paraganglioma (17). Another example is the nuclear-encoded mitochondrial enzyme fumarase (*FH*), an enzymatic component of the tricarboxylic acid cycle that catalyzes the formation of L-malate from fumarate. Mutations in the *FH* gene cause a predisposition to cutaneous and uterine leiomyomas, as well as kidney cancers (18). Finally, more recent studies have suggested that SNPs in the mitochondrial genome (mtSNPs) are associated with increased risk of several types of cancers, including invasive breast cancer in African American women (19) and prostate cancer (10,13,20).

The findings in previous studies suggest that genetic variations in mitochondria might play an important role in developing cancers, a hypothesis recently emphasized in a conference report (21). To systematically test the role of mitochondria in PC risk, we genotyped 24 SNPs from coding and regulatory regions in the mitochondrial genome and 376 tagSNPs in 78 nuclear-encoded mitochondrial genes among groups of cases and controls. The nuclear-encoded mitochondrial genes included 138 tagSNPs in 30 genes associated with mitochondria-related cancer pathways, 161 tagSNPs in 36 genes involved in mitochondrial respiratory chain and 77 tagSNPs in 12 genes serving as zinc transporters. Subjects included approximately 1000 PC cases, derived from men with familial PC (FPC), men with sporadic PC (SPC) and men with aggressive PC (APC) and 495 population controls.

Materials and Methods

Familial PC (FPC)

Ascertainment of families with PC has been described elsewhere (22). In brief,~ 200 high-risk families were identified following a survey of 12,675 men enrolled in the Mayo Clinic radical prostatectomy data base; families having a minimum of 3 men with PC were enrolled for further study. Blood was collected from as many family members as possible, resulting in a total of 498 affected men from 189 families. Of these, 490 affected men from 187 families are of non-Hispanic Caucasian ancestry. All men with PC who contributed a blood specimen had their cancers verified by review of medical records and pathologic confirmation. For the mitochondrial genome study, 435 affected men from 177 families were utilized for the analysis.

For nuclear-encoded mitochondrial gene analysis, we were able to genotype 395 men from 187 families, of which 355 were also included in the mitochondrial genome study. The research protocol and informed consent forms were approved by the Mayo Clinic institutional review board.

Sporadic PC (SPC)

Patients with SPC were selected from respondents to our family history survey who reported no family history of PC (23). To ensure that the SPC group was similar to the FPC group, except for family history, eligible patients with SPC were selected by frequency matching them to the FPC index patients according to year of diagnosis, age at diagnosis, and number of brothers. Blood samples were available for491 men of non-Hispanic Caucasian ancestry. All but10 of these men were treated surgically for their PC.

Aggressive PC (APC)

Patients with APC (Gleason grade ≥ 8) were also identified through the Mayo Clinic radical prostatectomy database. All men not previously contacted for our family history survey and who were diagnosed with high-grade PC were invited to participate. Of the 515 men eligible, 211 contributed a blood specimen and 204 were of non-Hispanic Caucasian ancestry. All of these men were treated surgically for their PC.

Population Controls

From a sampling frame of the local population provided by the Rochester Epidemiology Project (24), men were randomly selected for a clinical urologic examination (25). This examination included digital rectal examination (DRE) and transrectal ultrasound (TRUS) of the prostate, abdominal ultrasound for post void residual urine volume, measurement of serum levels of prostate-specific antigen(PSA) and creatinine, focused urologic physical examination, and cryopreservation of serum for subsequent sex hormone assays. Any patient with an abnormal DRE, elevated serum PSA level, or suspicious lesion on TRUS was evaluated for prostatic malignancy. If the DRE and TRUS were unremarkable but the serum PSA level was elevated (>4.0 ng/ml), then a sextant biopsy(three cores from each side) of the prostate was performed. An abnormal DRE or TRUS result, regardless of the serum PSA level, prompted a biopsy of the area in question. In addition, a sextant biopsy of the remaining prostate was performed. These men have been followed with biennial examinations. All men without PC on the basis of this work up and any follow-up exams were used in the control sample. The mitochondrial genome study included 490 population controls while the nuclear-encoded mitochondrial gene analysis included 495 population controls, 355 of who were in both control sets. All men are of non-Hispanic Caucasian ancestry.

Cancer Genetic Markers of Susceptibility (CGEMS) Data

After receiving approval to use the individual genotype data from the CGEMS phase I data (26), the genome-wide association data for 1,172 prostate cancer cases and 1,157 controls were downloaded from the website (http://cgems.cancer.gov/data).

SNP selection

Although thousands of mtSNPs have been reported (www.mitomap.org), the majority of these are rare (allele frequency < 1%). Based on allele frequency and the presence of common haplotypes in Caucasians (27), we selected 24 mtSNPs distributed across the mitochondrial genome for genotyping. Among these, 17 are within protein coding regions and the remaining 7 are in the regulatory region (displacement loop or D-loop). Ten of the 24 variants are SNPs that define common haplotypes in the Caucasian population (27).

There are over seven hundred known nuclear encoded mitochondrial proteins (www.mitoproteome.org). In this study, however, we focused our efforts on the three categories of proteins: a) respiratory chain proteins; b) mitochondria-related cancer proteins; and c) zinc transporter proteins. For these nuclear-encoded SNPs, our SNP selection relied on tagSNPs selected on the basis of linkage disequilibrium (LD) as implemented in ldSelect (28). To identify tagSNPs for each of the nuclear encoded genes selected for study, we used the publicly available genotype data from the HapMap Consortium based on NCBI build 35 assembly and dbSNP build 125. SNPs were binned using an r^2 threshold of 0.8. A set of tagSNPs were identified such that each exceeded this r^2 threshold with all other SNPs in the same bin. To choose between multiple tagSNPs within a bin, we implemented hierarchical selection criteria based on larger design scores provided by Illumina (San Diego, CA), greater minor allele frequency (MAF) and preference for coding over non-coding SNPs.

In this study, we selected only those genes that had ≥80% coverage by the LD bins. For example, if a gene extends from position 1 to 100, and contains one LD bin with markers ranging from position 1 to position 80, then that gene is described as having coverage of 80%. Additional 'singleton' SNPs within this gene, i.e., SNPs that are not in LD with any surrounding typed SNPs, do not increase the computed coverage, since they are not sufficiently correlated with any neighboring SNPs, although we recognize the possibility that these 'singleton' SNPs could be in LD with SNPs that are not in HapMap. We elected not to include singleton SNPs in our set of selected tagSNPs, focusing instead on those SNPs that are known to be in LD with at least one HapMap SNP. Of the 393 selected SNPs, 379 were successfully genotyped and 376 were of sufficient quality for further analysis. Two SNPs were excluded because the genotype frequencies in controls deviated from Hardy-Weinberg equilibrium (p-value < 0.001) and one SNP was removed due to poor clustering.

Genotyping for mtSNP

We used the Beckman SNPstream system for mtSNP genotyping. Two 12plex panels of primer sets were designed using the Web based Autoprimer. For each 12plex panel, 2ng DNA isolated from peripheral blood lymphocytes was amplified with the pooled primer sets (50nM each) under universal PCR conditions (5mM $MgCl_2$, 75 μM dNTPs, 0.1unit AmpliTaq Gold (Applied Biosystems, Foster City, CA)) in a final volume of 5 μl. After initial denaturation at 94°C for 1 minute, 34 cycles were performed at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The amplified materials were then cleaned by incubation with SBE Clean-up (shrimp alkaline phosphatase and exonuclease I) at 37°C for 30 minutes, and 96°C for 10 minutes. After 9 μl of extension mixes for each panel (C/T or G/A) was added to 7μl of cleaned PCR product, the plates were cylced at 96 °C for 3 minutes, then 45 cycles at 94 °C for 20 seconds, and 40°C for 11 seconds. SNPware array plates were prepared (washing with buffers I and II). Eight μl of hybridization solution was added to each well of the plate following primer extension reaction, and 10μl of this was added to the corresponding well in the SNPware tag plate, incubated at 42 °C and 100% humidity for 2 hours. The arrays were then washed and vacuum dried and imaged on the scanner. The SNPstream software was used for image data analysis and genotype calls. For each 384 well plate, quality control samples included 8 no-DNA template and 8 genomic controls. Final analysis revealed no signs of contamination or other technical problems associated with the genotype calls.

Genotyping for Nuclear gene SNP

We designed a custom GoldenGate oligonucleotide pool and used the Illumina platform to perform the genotyping (29,30). The majority of SNPs assayed had SNP design scores >0.6. Automated genotype clustering and calling was made using BeadStudio II. Summary and report files were generated within BeadStudio and transferred electronically to a server for analysis. Samples with GenCall scores below 0.25 and/or call rates below 95% were removed,

as were SNPs with GenCall scores below 0.4 and/or call rates below 95%. To confirm and refine clusters, we used 8 replicates of a CEPH family (parent-child trios). In addition, replicates of two DNA samples were included on each plate and 18 SNPs with known baseline genotype data on all cases and controls were included for quality control purposes. The error rate measured for the replicate samples and the 18 previously genotyped SNPs was 0.03%.

Statistical Analyses

Mitochondrial SNPs

Single SNP analysis—Although there are multiple mitochondrial DNA copies per cell, there is generally only a single allele for any given individual. In addition, mitochondria are transmitted maternally so that all FPC cases in the same pedigree with a common maternal ancestor will have identical alleles. We therefore identified independent FPC cases in the pedigrees by clustering subjects into groups defined by maternal ancestry and selecting a single subject per cluster. In this manner, 213 independent FPC cases were selected for analysis. The mtSNP allele frequency was used to assess the difference between the cases and controls using standard contingency 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. Odds ratios and 95% confidence intervals were computed for carriers of the minor allele versus subjects homozygous for the major allele.

Haplotype Analysis—Genotypes for 10 core mtSNPs that define common haplotypes in Caucasians (31) were combined to construct mitochondrial haplotypes. Haplotypes are specific combinations of nucleotides on the same mitochondrial genome. To test for an association between the mt-haplotype and case/control status, we calculated a score statistic using a modified version of the haplo-stats program (32) which implements an expectation–maximization (EM) algorithm to infer missing alleles for a haplotype. The analyses were based on global score statistics that compare all haplotypes between cases and controls.

Nuclear coding Mitochondrial SNPs

Single SNP analysis—For nuclear-encoded mitochondrial genes, single SNP genotype frequencies were compared between PC cases and controls using Armitage test for trend for the number of minor alleles. This coding assumes an additive model, with heterozygote risk intermediate between the two homozygotes. Permutation p-values based on 10,000 random simulations are reported, where case-control status was randomly permuted for each simulation. In order to account for the relatedness among FPC cases, a single FPC case was randomly selected from each pedigree.

Because testing was performed on a large number of SNPs, as well as multiple groups, both unadjusted p-values and those adjusted for multiple testing were computed. Adjustments were made separately for mitochondrial versus nuclear-encoded SNPs. Because some of these comparisons are dependent due to overlapping control group and SNPs in LD within a gene, the usual Bonferonni correction is too conservative. Hence, adjusted p-values were computed by 10,000 simulations. For each simulation, case-control status was randomly permuted and a new p-value was computed. The adjusted p-value was computed from the number of times out of 10,000 simulations that the minimum simulated p-value (over all SNPs and all group comparisons) was less than the observed p-value. These corrections accounted for the total number of SNPs evaluated, as well as the number of group comparisons (33).

Haplotype analyses—The number of SNPs studied per gene ranged from 1 to 28. Haplotype analyses were conducted using all of the tagSNPs within each gene when more than a single SNP was studied. Rare haplotypes (frequencies < 1%) were collapsed into a single haplotype

group, and the most frequent haplotype was considered the reference in the analyses. Global tests were conducted to assess the significance of all haplotypes simultaneously. In addition, the maximum of all haplotype specific tests, comparing each haplotype to the pool of all other haplotypes, was identified. Simulated p-values were used to avoid problems that may result from sparse data. In order to account for the relatedness among familial PC cases, we randomly selected a single case from each pedigree. This randomization was repeated 100 times and the average p-values are reported. Analyses were conducted using the haplo-stats package in Splus Version 8.0.1.

Principal Components—The haplotype analyses described above might not be optimal in the sense that analyses of genes with many SNPs compare many different haplotypes between cases and controls resulting in a global test with many degrees of freedom. As an alternative, we used principal components, a variable reduction procedure that typically results in a small number of components that account for most of the variance in a set of observed variables, in this case, the observed SNP genotypes within a candidate gene. The first N principal components that explained at least 90% of the variance in the observed SNPs were used to test for associations with PC. For each principal component, scores for each subject were calculated and these scores were used in a logistic regression model comparing all PC cases to controls. A global test for association was obtained for each gene with degrees of freedom equal to the number of principal component scores fit in the model. To account for the relatedness of the FPC cases, a single affected man was randomly selected from each pedigree to test for association. The process of randomly selecting FPC cases, computing the principal component scores and fitting logistic regression models was repeated 100 times. The average global p-value is reported.

Population Stratification—In order to investigate whether stratification exists among our patient groups due to differences in ethnic ancestry, we used all 376 nuclear encoded SNPs to create principal components. Plots of the first two principal components revealed random scatter for all four patient groups suggesting that population stratification is not likely to influence our results.

Results

The characteristics of the four study groups are shown in Table 1. The sets of FPC cases and controls used for each of the different studies are reported separately. Although the distribution of the age at diagnosis and body mass index (BMI) levels are similar between the FPC and SPC cases, the cases with APC and the controls tended to be younger and have higher BMI than the FPC and SPC cases. However, analyses adjusting for both age and BMI did not alter our findings. Therefore, for simplicity, we present only the unadjusted results.

We first evaluated mtSNPs and mitochondrial haplotypes for their association with PC utilizing 908 PC cases (213 cases from 177 FPC pedigrees, representing independent maternal clusters, 491 SPC and 204 APC) and 490 population-based controls. All 24 mtSNPs were genotyped in the FPC and SPC cases, as well as in the controls. Only 19 of the 24 mtSNPs were genotyped in the APC cases. The frequencies of these 24 mtSNPs along with odds ratios for the carriers of minor alleles, and their 95% confidence intervals are presented in Figure 1 (Supplementary Table 1). Overall, none of our findings were statistically significant after correcting for multiple comparisons, with all corrected p-values ≥ 0.95. Given the power limitations of this study, we used the CGEMS data as an independent follow-up data set to further evaluate our findings. Of the 24 mtSNPs tested, we found 12 identical SNPs in the CGEMS project data set. None of these 12 SNPs showed an association with prostate cancer in the CGEMS study.

To analyze mitochondrial haplotypes, we used 10 core mtSNPs that define common haplotypes in Caucasians: SNPs 1719, 4580, 7028, 8251, 9055, 10398, 12308, 13368, 13708, and 16391. To reduce computations, subjects who were missing 7 or more loci were excluded (2 controls, 22 SPC, and 7 APC). Eleven haplotypes with frequencies at least 1% were included in the analysis (Table 2). Based on a global score statistic that compares all 11 haplotypes between cases and controls, we did not identify any significant associations with FPC, SPC, APC or all cases combined - ALL PC (global p-values=0.091, 0.416, 0.604, and 0.499 respectively). When examined individually, two haplotypes showed suggestive associations with PC; the frequency of haplotype J was 14.6% in familial PC and 8.9% in controls (p=0.041) and the frequency of an unknown haplotype was 4.8% in sporadic PC, 4.3% in ALL PC and 1.5% in controls (p=0.027 and 0.022, respectively), but these p-values do not account for multiple testing.

We then evaluated 376 tagSNPs in 78 nuclear-encoded mitochondrial genes for their associations with PC utilizing 1,090 PC cases (395 men from 177 FPC pedigrees, 491 SPC and 204 APC) and 495 population-based controls. Table 3 shows the total number of known genes in each of the mitochondrial protein categories, the number of genes selected, selected SNPs, and successfully genotyped SNPs for each category (see Supplementary Table 2 for the gene list, coverage and tested SNPs/gene). No significant single SNP associations were detected after adjusting for multiple testing, with all corrected p-values ≥ 0.80. Considering all analyses performed [11 tests per SNP including all single-locus (three case groups plus ALL PC vs controls), haplotype (global and maximum haplotype specific tests for three case groups), and PCA models for a total of 2,256 total tests], 104 of 376 tagSNPs had at least one uncorrected p-value ≤ 0.05. These 104 SNPs included 39 of 138 mitochondria-related cancer pathways SNPs (15 genes), 48 of 161 mitochondrial respiratory chain SNPs (9 genes), and 18 of 77 zinc transporter SNPs (7 genes) (Supplementary Table 3).

As before, we used information derived from the CGEMS project data set to further investigate those tagSNPs demonstrating at least one uncorrected p-value ≤ 0.05. To compare the nuclear-encoded tagSNPs with the corresponding CGEMS data, we first matched the LD SNPs ($r^2 > 0.7$) between our study and the CGEMS study. Among the 104 tagSNPs in our study, 101 had at least one CGEMS SNP that was in high LD with our measured SNPs. Because most tagSNPs had multiple LD SNPs in the CGEMS data set (up to 14), we list only the CGEMS SNPs with the lowest p-values (Supplementary Table 4). Of the 104 tagSNPs described above, 7 also had a p-value < 0.05 in the initial phase of the CGEMS study. These 7 SNPs are located in five genes (*MTRR, NDUFA9, NDUFS2, NDUFB9* and *COX7A2*). A summary of the association results for the 7 CGEMS SNPs is shown in Table 4. Five of the seven SNPs were also included in the CGEMS phase II study, two of which had p-values < 0.10 in that follow-up analysis. However, for both of these SNPs the risk estimates were in opposite directions in the two studies.

Haplotype analysis was then performed for each of the nuclear encoded mitochondrial genes. For the gene *NDUFV2*, we observed a significant haplotype association with SPC (global p-value = 8.89×10^{-10}, simulated global p-value < 1/20,000) as well as marginal association with APC (simulated global p-value = 0.08). However, no association was observed with FPC (global p-value = 0.82) (Supplementary Table 5). Two haplotypes were associated with an increased risk of SPC; however, these same haplotypes were associated with a decreased risk of APC. The association of *NDUFV2* haplotypes with aggressive and non-aggressive PC was further explored using the CGEMS data. Our study includes five SNPs in the *NDUFV2* gene, only one of which was also genotyped in the CGEMS study. We inferred the four remaining SNPs in the CGEMS subjects using Mach, a Markov Chain based haplotyping method to infer missing genotypes in unrelated individuals (http://www.sph.umich.edu/csg/abecasis/MACH). This approach combined the sparser SNPs from the CGEMS data with the high-density SNPs of the HapMap CEU data, to use the LD in the CEU data to impute the unmeasured SNPs in

the CGEMS data. The imputation quality for the four SNPs was excellent, with individual SNP quality scores > 0.98 (on a scale of 0 – 1). Haplotype analyses were performed with CGEMS aggressive PC and nonaggressive PC cases resulting in global haplotype score statistic p-values of 0.14 and 0.06 respectively. The maximum haplotype specific score tests were also not statistically significant (p-values = 0.30 and 0.62 respectively) (Supplementary Table 5). Interestingly, the haplotype which had the strongest evidence for association with SPC and APC in the Mayo data was not observed in the CGEMS dataset, suggesting that the Mayo association is a chance finding.

Discussion

In this study, we tested the hypothesis that common variants in mitochondrial related genes are associated with an increased risk for PC. To accomplish this, we examined 24 SNPs in the mitochondrial genome, 11 mitochondrial haplotypes and 376 tagSNPs for nuclear-encoded mitochondrial genes for potential associations. There are over seven hundred known mitochondrial proteins (www.mitoproteome.org). In this study, however, we focused on the three categories of protein function: a) respiratory chain proteins, b) mitochondria-related cancer proteins and c) zinc transporter proteins. The respiratory chain proteins were chosen because some of these have been reported to cause hereditary cancer syndromes (34,35). Mitochondria-related cancer genes were identified through comparing lists of all nuclear-encoded mitochondrial genes (www.mitoproteome.org) to those of all cancer-related genes from a variety of databases. The zinc transporters were chosen because of the potential role of these proteins in the development and progression of prostate malignancy (36). Importantly, the SNPs chosen for these genes were selected to achieve greater than 80% coverage based on linkage disequilibrium. Overall, no statistically significant associations were detected for any of the SNPs individually or for the mtSNP haplotypes after adjusting for multiple comparisons. Our conclusions, however, are limited to relatively large effects of SNPs on prostate cancer risk. With approximately 490 cases and 490 controls, and correcting for approximately 1,600 statistical tests (across all SNPs and the four group comparisons), we had 85% power to detect an odds ratio of 2.5 if the risk allele has a population frequency of 10%, and 77% power to detect an odds ratio of 2.0 if the risk allele has a population frequency of 20%.

The CGEMS project, a collaborative whole genome association study initiated by National Cancer Institute, has the goal of identifying common genetic variations associated with risk for prostate and breast cancer (http://cgems.cancer.gov). The CGEMS project is projected to genotype ~ 8,000 prostate cancer cases over a three year period. The initial phase (Phase I) of the CGEMS study scanned over 550,000 tagSNPs in 1172 prostate cancer cases and 1157 controls. The replication phase (Phase II) of this study genotyped 26,958 selected SNPs in 3941 cases and 3964 controls. These association results, now publicly available, provides valuable information for further candidate gene selection, gene evaluation and replication of association results.

By taking advantage of this public data base, 12 of the 24 mtSNPs from the current study were also found to be present in the CGEMS data set. As with our results, a statistically significant association was not detected for any of these 12 mtSNPs in the CGEMS analysis. In addition to these 12 SNPs, 86 additional mtSNP were also tested in the CGEMS project. Of these, five demonstrated an incidence density adjusted score test p-value < 0.05. Given that approximately 550,000 SNPs were tested, these signals are not extreme enough to warrant statistical significance. Overall, the combined results suggest that common variants within the mitochondrial genome do not play a significant role in PC risk.

Our findings differ from those previously reported. Booker et al (20) compared mitochondrial haplotypes in a total of 121 PC cases, 221 renal cancer cases and 246 controls. They found that

mitochondrial haplotype U was a highly significant risk factor for prostate and renal cancer vs. controls (16.74% and 20.66% vs. 9.35%, p = 0.019 and 0.005, respectively). Since multiple haplotypes were analyzed and the sample size was relatively small, these findings most likely represent false positive associations. Of interest, the frequency of haplotype U among the controls in the current study is approximately 17%, a value closer to that reported for the cases in the Brooker study, suggesting that their control group is artificially low.

Of the 376 tagSNPs for the nuclear-encoded genes, 104 had at least one of eleven tests had unadjusted p-value ≤ 0.05. Recognizing that these findings are most likely due to chance (marginal p-values along with large number of statistical tests and modest sample size), we nevertheless further explored the significance of these findings by examining the data available through the CGEMS project. For these 104 tagSNPs, we identified 39 identical, 30 equivalent (based on LD, $r^2=1$) and 32 associated ($1 > r^2 \geq 0.7$) SNPs in the CGEMS Phase I database (3 tagSNPs had no corresponding CGEMS LD SNP). Among these 104 SNPs, seven CGEMS SNPs had an incidence density adjusted score test p-value less than 0.05. These seven CGEMS SNPs are located within 5 genes, four involved in the mitochondrial respiratory chain (*NDUFS2, COX7A2, NDUFB9, and NDUFA9*), and one in the mitochondria-related cancer pathway (*MTRR*). The three genes, *NDUFS2, NDUFB9* and *NDUFA9*, encode components of mitochondrial complex I. Both *NDUFB9 and NDUFA9* have NADH dehydrogenase and oxidoreductase activities. *COX7A2* is a subunit of cytochrome C oxidase, the terminal component of the mitochondrial respiratory chain that catalyzes the electron transfer from reduced cytochrome C to oxygen. The gene *MTRR* encodes a protein that regenerates a functional methionine synthase via reductive methylation. Because methionine is an essential amino acid required for protein synthesis and one carbon metabolism, polymorphisms in the gene have shown an association with various diseases including cancer (37–42). However, the seven CGEMS SNPs in the 5 genes are not located in coding regions or any other regulatory regions. Their functional consequences are not clear. Additionally, the five of these seven SNPs that were evaluated in the CGEMS phase II replication study did not have p-values < 0.05.

In summary, we did not find a significant role of mitochondrial SNPs in prostate cancer risk. For the 78 nuclear encoded mitochondrial genes tested, none of the tagSNPs were significant after correcting for multiple comparisons and none of the findings were replicated using the CGEMS data set.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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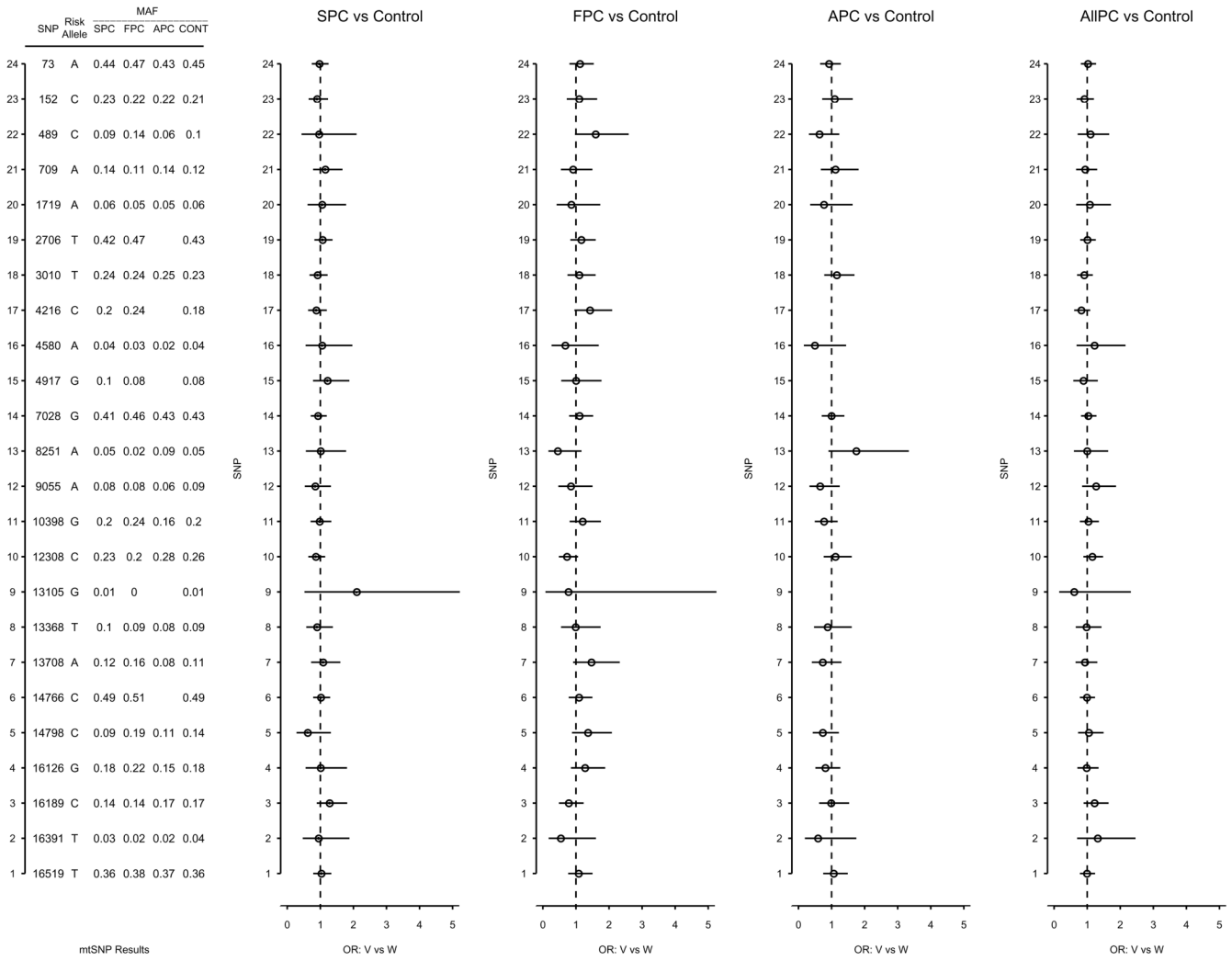

Figure 1.

Statistical analysis of mtSNPs and prostate cancer risk. Minor allele frequencies (MAF) of the 24 mtSNPs in four different populations were showed on left panel. Odds ratios for the carriers of minor alleles, and their 95% confidence intervals are displayed. SPC = sporadic PC, FPC = familial PC, APC = aggressive PC, AllPC = all PC cases, CONT = control. 5 mtSNPs were not genotyped in the cases with APC.

Table 1

Characteristics of Prostate Cancer Cases and Population Controls

			FPC[e]		Controls[f]	
	SPC N = 491	**APC N=204**	**Mitochondria SNPs[g] N=435**	**Nuclear Coding mtDNA SNPs N=395**	**Mitochondria SNPs N=490**	**Nuclear Coding mtDNA SNPs N=495**
Age[a], median (range)	65 (46–79)	64 (40–86)	66 (45–84)	65 (42–80)	63 (43–91)	63 (44–91)
Age[a], quartiles						
<= 59	90 (18)	59 (29)	66 (15)	86 (22)	205 (42)	186 (38)
60 – 65	175 (36)	53 (26)	128 (29)	131 (33)	84 (17)	102 (21)
66 – 70	167 (34)	47 (23)	131 (30)	117 (30)	68 (14)	83 (17)
> 70	59 (12)	44 (22)	110 (25)	61 (15)	133 (27)	124 (25)
Missing	0	1	0	0	0	0
Body Mass Index (kg/m^2; %)						
< 28	308 (63)	96 (49)	288 (67)	254 (64)	213 (43)	211 (43)
≥ 28	183 (37)	99 (51)	145 (33)	140 (36)	277 (57)	284 (57)
Missing	0	9	2	1	0	0
PSA (%)						
< 4	90 (23)	26 (17)	40 (12)	40 (13)	444 (91)	457 (92)
4 – 9.9	173 (44)	74 (50)	153 (46)	138 (46)	42 (9)	35 (7)
10 – 19.9	72 (18)	28 (19)	68 (20)	62 (20)	3(1)	3 (1)
≥ 20	61 (15)	21 (14)	72 (22)	62 (20)	1(0)	0 (0)
Unknown	95	55	102	93	0	0
Pathologic Characteristics (%):						
Nodal Status[b]						
Negative	428 (88)	152 (85)	299 (86)	283 (87)		
Positive	57 (12)	27 (15)	50 (14)	43 (13)		
Unknown	6	25	86	69		
Stage[c]						
1/2	287 (67)	62 (40)	268 (71)	229 (69)		
3/4	139 (33)	92 (60)	108 (29)	102 (31)		
Unknown	8	23	9	21		
Gleason Score[d]						

			FPC[e]		Controls[f]	
	SPC N = 491	**APC N=204**	**Mitochondria SNPs[g] N=435**	**Nuclear Coding mtDNA SNPs N=395**	**Mitochondria SNPs N=490**	**Nuclear Coding mtDNA SNPs N=495**
< 7	263 (67)		249 (70)	215 (68)		
≥ 7	127 (33)		108 (30)	102 (32)		
Unknown	101		78	78		

[a] Defined as Age at diagnosis for PC patients, and age at last follow-up for Controls.

[b] Nodal Status positive group includes subjects who had metastatic disease.

[c] Subjects whose nodal status was positive are excluded.

[d] All APC have Gleason Score ≥ 8 (99 grade 8, 95 grade 9, 10 grade 10)

[e] The 435 FPC cases included in mitochondria SNP analysis are from 187 families; The 395 men used for NuclearCoding mtDNA SNP association are from 177 families. The two familial cases groups have 355 men in common.

[f] The two control groups have 380 men in common.

[g] These 435 men represent 213 independent subjects defined by clustering men based on maternal lineage.

Table 2

Ten Common Haplotypes in Prostate Cancer Patients

mtSNPs	1719	4580	7028	8251	9055	10398	12308	13368	13708	16391	Haplotype Frequencies					p-value			
											Cont	SPC	FPC	APC	All PC	SPC vs Cont	FPC vs Cont	APC vs Cont	All PC vs Cont
Haplo-groups																			
I	A	G	A	A	G	G	T	C	G	T	0.031	0.030	0.019	0.021	0.025	0.952	0.494	0.588	0.584
X	A	G	A	G	G	A	T	C	G	C	0.019	0.007	0.019	0.010	0.010	0.133	0.968	0.552	0.218
V	G	A	A	G	G	A	T	C	G	C	0.038	0.037	0.028	0.021	0.031	0.946	0.565	0.287	0.529
W	G	G	A	A	G	A	T	C	G	C	0.012	0.007	NA	0.033	0.011	0.488	0.247	0.235	0.825
U1	G	G	A	G	A	A	C	C	G	C	0.017	0.016	0.019	0.016	0.016	0.927	0.877	0.954	0.989
K	G	G	A	G	A	G	C	C	G	C	0.068	0.060	0.061	0.052	0.058	0.626	0.754	0.456	0.487
U2	G	G	A	G	G	A	C	C	G	C	0.162	0.150	0.115	0.202	0.153	0.638	0.093	0.223	0.692
Unknown	G	G	A	G	G	A	T	C	G	C	0.015	0.048	0.034	0.039	0.043	**0.027**	0.316	0.217	**0.022**
T	G	G	A	G	G	A	T	T	G	C	0.082	0.094	0.086	0.078	0.089	0.521	0.874	0.852	0.691
J	G	G	A	G	G	G	T	C	A	C	0.089	0.099	0.146	0.083	0.107	0.610	**0.041**	0.777	0.313
H	G	G	G	G	G	A	T	C	G	C	0.412	0.391	0.446	0.404	0.407	0.495	0.396	0.854	0.864
														Global p-value		0.416	0.091	0.604	0.499

The letters preceding each haplotype correspond to the haplotype labels in the paper “Mitochondrial Polymorphisms Significantly Reduce the Risk of Parkinson Disease”, Am. J. Hum. Genet. 72:804–811, 2003. One of the haplotypes above does not fit any from the paper. Also, two of the above (U1 and U2) both meet the description of haplotype U in the paper.

Table 3

Summary of gene categories and tagSNP selection

	# of Genes	# of Selected Genes	# of Selected tagSNPs	# of Successfully Genotyped tagSNPs (Genes) †	# of tagSNPs (Genes) that passed QC filters‡
Mitochondrial Genome	-	-	24	24	24
Nuclear-encoded Genes					
Electron Transport Chain	85	46	176	163 (36)	161 (36)
Cancer-related	84	32	141	139 (30)	138 (30)
Zinc Transporters	24	16	81	77 (12)	77 (12)
Total	193	94	393	379 (78)	376 (78)

† 19 SNPs were not successfully genotyped; 11 SNPs were dropped and 8 were excluded due to atypical clustering

‡ 3 additional SNPs were excluded for QC reasons: 1 due to atypical clustering and 2 due to deviation from HWE (p-value < 0.001).

Table 4

CGEMS Phase I and II study analysis of Mayo SNPs of interest

		Mayo		CGEMS PhaseI				
Gene; Chrom	SNP	Minor Allele (MAF)	OR (95% CI) †	CGEMS SNP (r^2)	Minor Allele (MAF)	p-value‡	OR (95% CI)† NonAggrPC	OR (95% CI)† AggrPC
Mitochondrial respiratory chain								
NDUFS2; 1	rs4233367	A (0.41)	0.90 (0.77, 1.05)	rs10797094 (0.90)	G (0.43)	0.007	0.97 (0.83,1.13)	1.00 (0.87,1.14)
	rs2070902	T (0.24)	1.01 (0.84, 1.22)	rs2070902	T (0.24)	0.036	1.02 (0.86,1.22)	1.07 (0.91,1.25)
COX7A2; 6	rs1203213	A (0.05)	1.41 (0.98, 2.04)	rs638590 (1.0)	C (0.04)	0.004	1.73 (1.25,2.40)	1.08 (0.78,1.51)
NDUFB9; 8	rs3829037	G (0.23)	1.26 (1.05, 1.52)	rs3829037	G (0.23)	0.033	0.73 (0.61,0.89)	0.99 (0.85,1.16)
	rs7830235	A (0.13)	1.07 (0.84, 1.36)	rs17299208 (0.93)	A (0.12)	0.034	0.64 (0.49,0.84)	0.95 (0.77,1.17)
NDUFA9; 12	rs4147684	A (0.27)	0.94 (0.79, 1.12)	rs7972920 (1.0)	A (0.29)	0.015	0.89 (0.75,1.06)	0.79 (0.68,0.92)
Mitochondria—related cancer								
MTRR; 5	rs17184211	A (0.20)	0.95 (0.78, 1.15)	rs17243407 (1.0)	A (0.19)	0.020	1.20 (0.99,1.45)	1.07 (0.90,1.28)

† Odds ratios (OR) and their 95% confidence intervals (95% CI) are estimated using a logistic regression model assuming an ordinal (log-additive) genotypic effect on PC risk. For the Mayo analyses the reported OR (95% CI) are for all PC cases versus controls.

‡ P-value from CGEMS Phase I whole genome association study. The incidence-density sampling adjusted score test p-value is reported.

§ P-value from CGEMS Phase II Replication dataset. The minimum adjusted p-value over four genetic models tested (dominant, recessive, additive, and genotype trend) and four phenotype definitions (dichotomous phenotype: Non-aggressive PC vs controls, Aggressive PC vs controls, and All PC vs controls; and trichotomous phenotype) is reported.