

Sequence Variation in the Mitochondrial Gene Cytochrome *c* Oxidase Subunit I and Prostate Cancer in African American Men

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BACKGROUND. Previous studies have found associations between mitochondrial DNA (mtDNA) mutations and several cancer types. Recently, we found that mutations in the mtDNA gene cytochrome *c* oxidase subunit 1 (*COI*) were both linked to and associated with prostate cancer (PCa) in Caucasian men. Here we examine the association between *COI* mutations and PCa in African American men.

METHODS. The entire *COI* gene was directly sequenced in 132 PCa cases and 135 controls from the Flint Men's Health Study, a community-based sample of African American men with and without PCa. Associations between all variants and PCa were evaluated.

RESULTS. We identified 102 *COI* single nucleotide polymorphisms (SNPs), including 15 missense variants. Overall, the presence of one or more *COI* missense variants was not significantly associated with PCa. Individually, two SNPs (T6221C and T7389C) were significantly associated with prostate cancer ($P < 0.05$) and in strong linkage disequilibrium with each other ($r^2 > 0.6$).

CONCLUSIONS. Of the two significantly associated SNPs, one is a synonymous substitution and the other is part of the African-specific mitochondrial haplogroup (L). Additional research will be needed to determine the clinical relevance of these associations in African populations.

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KEY WORDS: prostate cancer; *COI*; SNP; association

INTRODUCTION

Prostate cancer (PCa) is the most frequently diagnosed cancer, and the second leading cause of cancer death among American men [1]. Positive family history and African American ancestry are two of the most important recognized risk factors for PCa. Compared to Caucasian men, African American men are 1.6 times more likely to be diagnosed with PCa and 2.4 times more likely to die from the disease [1]. Although the reasons for this disparity are unknown, genetic factors may play an important role.

Anna M. Ray and Kimberly A. Zuhlke contributed equally to this work.

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Mitochondrial (mtDNA) gene mutations have been associated with several types of cancer, including PCa [2,3]. These mutations may play a role in cancer formation by increasing the production of reactive oxygen species (ROS) during mitochondrial oxidative phosphorylation. The resultant ROS are mitogenic, and therefore have functional relevance in cancer proliferation. Mitochondrial mutations can lead to an increase in ROS production, which can in turn lead to tumorigenesis and increased tumor growth. For example, a mutation in the mtDNA gene *ATP6* has been shown to increase ROS production and promote growth of PC3 PCa cells in vivo [4]. In addition, increased intracellular ROS caused by mtDNA mutations can mediate tumor metastasis in vivo [5]. Others have demonstrated altered expression of transcripts from mitochondrial genes in PCa tissue compared to paired normal tissue [6]. Using quantitative real-time PCR, Mizumachi et al. [7] identified a higher cellular content of mtDNA in PCa cells compared to adjacent normal prostate cells, suggesting another mechanism in which mtDNA may play a role in PCa carcinogenesis.

We previously reported that germline mutations in the mitochondrial gene encoding cytochrome *c* oxidase subunit 1 (*COI*) are associated with PCa [4]. In that report, we focused primarily on germline and tumor DNA samples from Caucasian men. Because of the importance of PCa in African American men, we set out to test the hypothesis that germline *COI* mutations are more common in African American men with PCa (vs. without) in the Flint Men's Health Study (FMHS) [8], a community-based case-control study of PCa in African American men.

MATERIALS AND METHODS

Subjects

Subjects selected from the FMHS submitted an informed consent. All documents and protocols were reviewed by the Institutional Review Board at the University of Michigan Medical School. As described previously [8], FMHS controls were recruited between 1996 and 1998 from a probability sample of African American men between the ages of 40 and 79 years living in Genesee County, MI. Control subjects completed a detailed epidemiologic interview and a PCa screening protocol consisting of a serum total prostate-specific antigen (PSA) measurement and a urologic examination. Men with elevated PSA (defined as ≥ 4 ng/ml) and/or abnormal digital rectal exam (DRE) were referred for biopsy. Control men were recontacted for a second interview ~ 5 years after the first interview. If a control received a diagnosis of PCa either through clinical care or through participation in the FMHS study, he was considered to be a case.

PCa case recruitment from the same community was initiated in 1999 and completed in July 2002. Participation of cases required: (1) an epidemiologic interview, (2) a review of the hospital and registry records for information on tumor stage, Gleason score, pre-diagnosis PSA, and type of therapy, and (3) provision of a blood sample for DNA, serum, and plasma. Genomic DNA was obtained from whole blood using a Puregene DNA extraction kit (Gentra Systems, Minneapolis, MN).

COI Sequencing

The *COI* gene was PCR amplified for all 132 FMHS cases with available DNA and the 135 oldest control men who had a total serum PSA < 4 ng/ml at the time of the first FMHS blood draw. One control sample was found to exhibit a significant degree of heteroplasmy at several locations and was consequently removed from future analyses. Primer sequences were previously published [4], and primers were purchased from Invitrogen Life Technologies (Carlsbad, CA). PCR products were purified using Montage PCR Centrifugal Filter Devices (Millipore, Billerica, MA). Cycle sequencing reactions were performed in both forward and reverse directions using Big Dye Terminator v3.1 Chemistry (Applied Biosystems, Foster City, CA), and excess dye terminators were removed using Performa DTR Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, MD). Cycle sequencing products were sequenced using an ABI 3100 Genetic Analyzer. Polymorphisms were identified with Mutation Surveyor 2.51 software (Soft Genetics, State College, PA).

Statistical Analysis

Linkage disequilibrium between pairs of single nucleotide polymorphisms (SNPs) was estimated using the r^2 measure. Due to low minor allele frequencies for some SNPs, we used Fisher's exact test to evaluate the association between each SNP and PCa. For SNPs with five or more copies of the minor allele present in both cases and controls, we also used logistic regression to test for the association between each SNP and PCa, including age in the model as a potential confounder.

To test for population substructure between the cases and controls, we implemented the method of Pritchard and Rosenberg [9] using 42 unlinked microsatellite markers. The observed summary chi-square measure was 117.73 with 142 degrees of freedom (P -value = 0.93), suggesting that evidence of population substructure was not detectable in our sample. All analyses were carried out using the R language (version 2.1.1), and all statistical tests were one-sided and based on a significance level of 0.05.

TABLE I. Characteristics of Men With PCa (n = 132)

Trait	n ^a	(%)
Age at diagnosis ^b	63	[56–69]
Prediagnosis PSA level ^b	6.5	[4.3–11.9]
Surgery ^c	73	(56.2)
Stage		
Localized	99	(76.7)
Locally advanced	24	(18.6)
Metastasized	6	(4.7)
Gleason		
≤6	37	(28.9)
7	76	(59.4)
>7	15	(11.7)
Clinically aggressive PCa		
Yes	48	(36.4)
No	84	(63.6)

^aColumn subtotals that do not sum to 132 are due to missing data.

^bMedian and [interquartile range] are reported.

^cNumber and percentage of men with PCa who underwent a radical prostatectomy.

RESULTS

The average age of PCa diagnosis for the 132 cases was 63 years (interquartile range of 56–69 years). The distribution of Gleason grade for these cases was as follows: 29% Gleason <7, 59% Gleason =7, and 12% Gleason >7 (Table I). More than half of cases (56%) underwent radical prostatectomy as part of their primary treatment for PCa. For the 135 control men, the average age at initial consent was 65 years

(interquartile range of 60–70 years), with a mean PSA of 1.29 ng/ml (interquartile range of 0.61–1.8 ng/ml).

The entire coding region of the *COI* gene (nucleotides 5904–7445) was sequenced using genomic DNA from each of the 132 cases and 135 controls. A total of 102 *COI* variants were identified in the amplified region, including 15 missense variants (Table II). Overall, more *COI* missense variants were identified in cases compared to controls, although the difference was not statistically significant (43% vs. 33%, $P = 0.10$, Table III). Individually, nine variants exhibited allele frequency differences greater than 5%, and each of the variants was more common in cases than in controls (Table IV). Two of these variants (T6221 and T7389C) were significantly more frequent in cases than in controls. The T7389C variant has been previously been shown to define the L mitochondrial haplogroup, a specific marker of African ancestry [10], and was in strong linkage disequilibrium with T6221C (r^2 of 0.81 in cases and 0.62 in controls).

DISCUSSION

We first described the association between germline mtDNA mutations and PCa several years ago [4]. In that study, we observed that *COI* missense mutations were more common in Caucasian men with PCa than in Caucasian men who had been screened with serum PSA and prostate biopsy and found not to have PCa (11.9% vs. 1.9%). Additional findings in that study included *COI* missense mutations in a population of 1,338 individuals not selected for PCa status and found

TABLE II. COI Missense Variants in Cases and Controls

Nucleotide	Amino acid	Controls (n = 135)		Cases (n = 132)	
		Count	%	Count	%
C5911T*	A3V	4	3.0	2	1.5
A6040G	N46S	0	0.0	1	0.8
G6150A*	V83I	8	5.9	4	3.0
T6253C*	M117T	7	5.2	4	3.0
G6261A*	A120T	1	0.7	1	0.8
G6366A	V155I	1	0.7	1	0.8
G6480A	V193I	0	0.0	2	1.5
A6663G*	I254V	6	4.4	8	6.1
A6891G	S330G	0	0.0	1	0.8
A7146G	T415A	34	25.2	42	31.8
A7158G*	I419V	0	0.0	2	1.5
A7299G	M466V	1	0.7	0	0.0
T7354C	M484T	1	0.7	0	0.0
T7389C	Y496H	24	17.8	36	27.3
G7444A	X514K	1	0.7	0	0.0
Total		88	4.4	105	5.3

TABLE III. Number of COI Missense Variants Per Individual

Number of variants	Controls (n = 135)		Cases (n = 132)	
	Count	%	Count	%
0	90	66.7	75	56.8
1	17	12.6	19	14.4
2	20	14.8	34	25.8
3	1	0.7	0	0.0
4	7	5.2	3	2.3
5	0	0.0	1	0.8
Total with ≥ 1 variant	45	33.3	57	43.2

that amongst these "population controls," mutation rates were 7.8% overall, including 6.5% in Caucasians and 17.4% in African American men. Silent mutations were not reported. In the current study, we have reported all DNA base changes including silent and missense (amino acid altering). While it is possible that a silent mutation could affect cellular physiology, there is no evidence that this occurs in the mitochondrial genome. Thus, of the two mutations found to be associated with PCa in this study (T6221C and T7389C) the silent mutation (T6221C) is certainly less likely to be causally related to PCa than the missense mutation (T7389C). To our knowledge, there are no other reports that focus on the relationship between COI mutations and PCa in African American men. For this investigation, we hypothesized that missense mutations in the COI gene would be more common in African American PCa cases than in African American controls.

Overall, we did not find a significant association between the presence of one or more COI missense variants and PCa, although the frequency of COI

variants was greater in cases (43.2%) than in controls (33.3%). We identified 15 COI missense variants in our sample, including 9 that were not discovered in our previous study of Caucasian men. Among the SNPs with allele frequency differences $>5\%$ between cases and controls, two (T6221C and T7389C) were significantly associated with PCa and in strong linkage disequilibrium ($r^2 > 0.6$) in our sample. T6221C is a synonymous mutation and therefore unlikely to result in a functional alteration in COI activity. Although T7389C alters an amino acid and therefore could be deleterious to COI function, it is part of the African mitochondrial haplogroup L (specifically the L1b and L1C subclusters) [11], making it difficult to determine its contribution to PCa risk in our sample. This mutation has also occurred as a somatic mutation in papillary thyroid carcinoma [12] and medulloblastoma [13]. Wang et al. [14] recently reported a comprehensive analysis of mitochondrial SNPs in a set of Caucasian PCa cases and controls. Only one SNP in COI at position 7028 was examined, and no association was detected between this SNP and PCa. Since this variant marks a Caucasian haplotype, we detected the risk allele (G) in only 2/267 African American men in FMHS (0 cases and 2 controls).

Unlike a previous study of Caucasian men [4], we did not find compelling evidence that COI mutations play a role in PCa risk in African American men. Further research in African populations will be necessary to tease out the potential significance of the T6221C and T7389C mutations in African American PCa.

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TABLE IV. SNPs With $>5\%$ Difference in Allele Frequency Between Cases and Controls

SNP ^a	Controls (n = 135)		Cases (n = 132)		% difference	P-value ^b
	Count	%	Count	%		
A5951G	11	8.2	18	13.6	5.4	0.10
T6071C	11	8.2	18	13.6	5.4	0.10
T6221C	13	9.6	26	19.7	10.1	0.02
A7055G	22	16.3	29	22.0	5.7	0.11
A7146G	34	25.2	42	31.8	6.6	0.10
T7175C	21	15.6	29	22.0	6.4	0.13
C7256T	74	54.8	83	62.9	8.1	0.15
C7274T	21	15.6	29	22.0	6.4	0.13
T7389C	24	17.8	36	27.3	9.5	0.03

^aAll SNPs are silent except, A7146G and T7389C.

^bP-values were one-sided and calculated based on a logistic regression model adjusting for age.

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