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SHORT COMMUNICATION

No association between typical European mitochondrial variation and prostate cancer risk in a Spanish cohort

Laura Fachal[1], Antonio Gómez-Caamaño[2], Vanesa Álvarez Iglesias[3], Alberto Gómez Carballa[3], Patricia Calvo[2], Antonio Salas[3,4] and Ana Vega[1,4]

Mitochondrial common variants (mtSNPs) and the haplogroups defined by them have been inconsistently correlated with increased prostate cancer risk. Here we aimed to investigate the influence of the mitochondrial genetic background on prostate cancer. A total of 15 single-nucleotide polymorphisms (SNPs) representing the common European branches of the mtDNA phylogeny were analyzed in a cohort of 620 Spanish prostate cancer patients and 616 matched population-based controls. Association tests were computed on mtSNPs and haplogroups. None of the evaluated mtSNPs or haplogroups were statistically associated with prostate cancer risk in our Spanish cohort. We show that previous association findings do not rest on solid grounds given that all of them (i) were based on underpowered studies, (ii) did not control for population stratification, (iii) lacked replication/confirmation cohorts, and (iv) and did not control for multiple test corrections. Taken together, a critical reassessment of the previous literature and the results obtained in the present study suggest that mtDNA common European variants are not correlated with increases in the risk for prostate cancer.
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INTRODUCTION

Mitochondrial germline DNA variation has been suggested as a marker of susceptibility to numerous types of cancer given the role of mitochondria as the major source of reactive oxygen species production and as part of the apoptosis system. Several studies have inconsistently reported an association between DNA single-nucleotide polymorphisms (mtSNPs) and common haplogroups with prostate cancer susceptibility.[1–7] Here we aimed to (i) assess the potential pathogenic role of well-known mtDNA variants, and the haplogroups defined by these mtSNPs, in the risk of developing prostate cancer and (ii) validate positive associated mtSNPs identified in cohorts of prostate cancer patients by others.

MATERIALS AND METHODS

Study subjects

Samples were obtained from 620 unselected consecutive Galician (NW Spain) prostate cancer patients, treated as previously described.[8] The data have been generated previously and analyzed in the context of radio-induced therapy.[9] Clinical characteristics of the patients are summarized in Table 1. We have now carried out a case–control study of these prostate cancer patients with an ethnically matched Spanish group that consisted of 616 individuals; these controls corresponded to the CG2 group analyzed previously.[10]

Written informed consent was obtained for each subject according to the protocols approved by the ethics review board of the Galician Ethical Committee for Clinical Research and in compliance with the declaration of Helsinki principles.

mtSNP selection and genotyping

mtDNA SNP selection was carried out as in the study by Salas *et al.*[10] A set of 15 mtSNPs representing the most common European branches of the phylogeny were genotyped as described in the study by Cerezo *et al.*[11] mtDNA haplotypes were phylogenetically checked following the methodology reported by Salas and colleagues.[12] Figure 1 in the study by Fachal *et al.*[9] shows the phylogeny that indicates the correspondence between the mtSNPs analyzed in the present study and the main European haplogroups they represent.

Statistical analysis

Association analyses for individual mtSNPs as well as for individual haplogroups with prostate cancer were performed using a one-degree of freedom Pearson's χ^2-test or, when appropriate, Fisher's exact test. Statistical analyses were carried out using R v2.15.2 (http://www.r-project.org/). MitPower[13]

[1]Fundación Pública Galega de Medicina Xenómica-SERGAS, Grupo de Medicina Xenómica, CIBERER, IDIS, Santiago de Compostela, Spain; [2]Department of Radiation Oncology, Complexo Hospitalario Universitario de Santiago, SERGAS, Santiago de Compostela, Spain and [3]Unidade de Xenética, Facultade de Medicina, Departamento de Anatomía Patolóxica e Ciencias Forenses, Instituto de Ciencias Forenses, Universidade de Santiago de Compostela, Galicia, Spain
[4]These authors contributed equally to this work.
Correspondence: Professor A Salas, Unidade de Xenética, Facultade de Medicina, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Universidade de Santiago de Compostela, 15872 Galicia, Spain.
E-mail: antonio.salas@usc.es
or Dr A Vega, Fundación Pública Galega de Medicina Xenómica-SERGAS, Grupo de Medicina Xenómica, Edificio de Consultas, CIBERER, IDIS, Planta -2, Choupana s/n, 15706 Santiago de Compostela, A Coruña, Spain.
E-mail: ana.vega@usc.es
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Table 1 Clinical characteristics of the patients

	N *(%)*	
Age[a] mean (range)	70	(46–81)
PSA mean (range)	15.58	(0.63–263)
Gleason Score[a] (range)	6.10	(2–10)
Clinical T stage		
cT1	209 (33.71)	
cT1b		3 (0.48)
cT1c		206 (33.23)
cT2	335 (54.03)	
cT2a		52 (8.39)
cT2b		169 (27.26)
cT2c		113 (18.23)
cT2x		1 (0.16)
cT3	46 (7.42)	
cT3a		11 (1.77)
cT3b		14 (2.26)
cT3x		21 (3.39)
cT4	8 (1.29)	
Missing	22 (3.55)	
N stage[a]		
N0	538 (86.77)	
N1	11 (1.77)	
Nx	51 (8.23)	
Missing	20 (3.23)	
M stage[a]		
M0	542 (87.42)	
M1	3 (0.48)	
Mx	55 (8.87)	
Missing	20 (3.23)	

Abbreviation: PSA, prostate-specific antigen.
[a]At diagnosis.

(http://bioinformatics.cesga.es/mitpower/) was used for estimation of the statistical power. Meta-analysis of haplogroup U results was carried out with the R library *meta*, using fixed- and random-effects models and the inverse variance method for pooling the effects (odds ratios) observed in each study.

RESULTS

Association tests were carried out individually for mtSNPs and mtDNA haplogroups (Table 2). We did not find a statistically significant association between haplogroup and mtSNP distribution in prostate cancer patients compared with the control group. The statistical power to detect increases in prostate cancer risk higher than 1.75 was 83.1 (Figure 1). It is important to note that the present work showed the highest statistical power among the different studies published to date on prostate cancer and mtDNA variants (Table 3). The results of the meta-analysis carried out on haplogroup U are shown in Figure 2. Statistically significant heterogeneity between the studies was observed ($I^2 = 0.62$, P-value $= 0.022$). However, no association between haplogroup U and prostate cancer risk could be observed with any of the models used (fixed effects P-value $= 0.32$, random effects P-value $= 0.21$).

DISCUSSION

The aim of the present study was to evaluate the influence of mtSNPs and haplogroups in prostate carcinogenesis by means of a case–control study involving 1236 individuals. Previous mtDNA

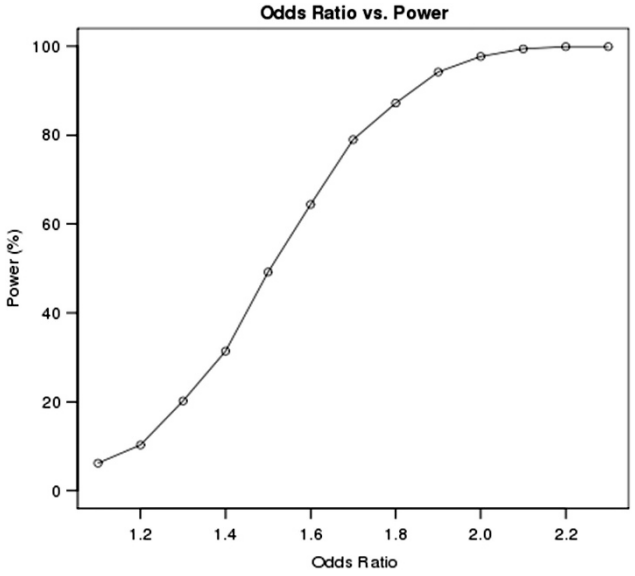

Figure 1 Power estimates in our case–control association study were determined using the software mitPower. Statistical power was computed using Fisher's Exact Test (1000 Monte Carlo simulations) and the following parameters: number of categories (mtSNPs or haplogroups) = 15; frequency for the risk allele = 0.24; range of odds ratios from 1.1 to 2.3 in increments of 0.01. The frequency of the risk allele is the frequency of haplogroup U in our sample (see Table 3).

association studies on prostate cancer have shown contradictory findings.[1–7] Three of them showed positive associations. Ray *et al.*[2] indicated the association of T6221C and T7389C mtSNPs in prostate cancer, whereas Booker *et al.*[4] and Canter *et al.*[6] showed the association of haplogroup U. Note, however, that these three studies were underpowered when considering odds ratio values $\leqslant 2$ (Table 3). Besides, none of these positive studies provides control for multiple testing (with the exception of the study by Canter *et al.*,[6] in which only one mtSNP was evaluated). We investigated the literature for other prostate cancer studies in which these SNPs and haplogroups were tested and found four studies indicating the lack of association between haplogroup U and prostate cancer.[1,3,5,7] However, apart from the study by Wang *et al.*,[7] none of the other three studies are powered enough to detect at least twofold increases in risk. In order to increase the power to detect a putative effect of haplogroup U in prostate cancer risk we have meta-analyzed the results from previous studies. No statistical significance was observed. Regarding associated mtSNPs from the study by Ray *et al.*,[2] to our knowledge, neither T6221C nor T7389C mtSNP was interrogated in relation to prostate cancer susceptibility by other studies.

Among the limitations of our study is the fact that we have analyzed 15 mtSNPs signaling well-known European haplogroups, and therefore we cannot exclude the possibility of other variations being associated with prostate cancer risk. For instance, we did not explore variation within some of the main haplogroups tested in the present study (see Phylotree Build 16 for a refined version of the worldwide mtDNA phylogeny; www.phylotree.org); some of them are also frequent in the Spanish population. Furthermore, our study is not powered enough (that is, above 80%) to detect increases in risk lower than 1.75.

In summary, we did not observe statistical association between the mtDNA variants and haplogroups tested in our study with prostate cancer. Our study confirms other's findings (although underpowered)

Table 2 Association test between mitochondrial tSNPs and haplogroups with prostate cancer

	Cases		*Controls*				
Variant	*Frequency*	N	*Frequency*	N	*OR*[a]	*95% CI*	P-*value*
SNP [b]							
T3197C (C)	0.10	620	0.10	607	1.02	0.71–1.48	0.88[c]
T4216C (C)	0.15	620	0.16	611	0.97	0.71–1.32	0.85[c]
A4529T (T)	4.84×10^{-3}	620	1.63×10^{-3}	614	2.98	0.31–28.73	0.62[d]
G4580A (A)	0.03	620	0.03	616	1.04	0.56–1.95	0.89[c]
C7028T (C)	0.46	620	0.45	615	1.06	0.85–1.32	0.62[c]
G8994A (A)	0.02	617	0.02	613	1.19	0.51–2.79	0.68[c]
A10398G (G)	0.18	600	0.20	613	0.82	0.61–1.10	0.19[c]
T10463C (C)	0.09	600	0.09	607	1.03	0.69–1.53	0.87[c]
T10873C (C)	0.04	620	0.04	613	1.09	0.57–1.85	0.91[c]
G11719A (A)	0.46	618	0.47	607	0.96	0.77–1.20	0.72[c]
A12308G (G)	0.24	620	0.24	609	0.97	0.74–1.26	0.81[c]
C12705T (T)	0.08	620	0.07	616	1.09	0.71–1.67	0.69[c]
G13708A (A)	0.07	620	0.09	607	0.77	0.52–1.16	0.21[c]
A13966G (G)	0.01	618	0.02	613	0.49	0.19–1.22	0.12[c]
C14766T (T)	0.47	620	0.48	614	0.94	0.76–1.18	0.61[c]
HG							
H	0.46	620	0.45	616	1.07	0.85–1.34	0.56[c]
HV	0.53	620	0.51	616	1.07	0.86–1.64	0.53[c]
I	4.84×10^{-3}	620	1.62×10^{-3}	616	2.99	0.31–28.82	0.62[d]
J	0.06	620	0.07	616	0.74	0.47–1.78	0.20[c]
K	0.06	620	0.09	616	0.70	0.46–1.07	0.10[c]
K1	0.06	620	0.08	616	0.77	0.50–1.20	0.25[c]
R	0.54	620	0.52	616	0.92	0.60–1.40	0.65[c]
R0	0.92	620	0.93	616	1.05	0.84–1.34	0.69[c]
T	0.09	620	0.08	616	1.06	0.71–1.58	0.79[c]
TJ	0.14	620	0.16	616	0.90	0.66–1.22	0.49[c]
U	0.24	620	0.24	616	0.98	0.76–1.28	0.90[c]
U5	0.10	620	0.10	616	1.05	0.72–1.51	0.81[c]
V	0.03	620	0.03	616	1.04	0.56–1.94	0.89[c]
W	0.01	620	0.01	616	1.14	0.41–3.16	0.80[c]
X	0.01	620	0.02	616	0.59	0.21–1.64	0.31[c]

Abbreviations: 95% CI, 95% confidence interval; HG, haplogroup; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]ORs are referred to the minor allele or the HG status.
[b]In round parentheses are the alleles with the lowest frequency.
[c]Pearsońs χ^2-test *P*-value.
[d]Fisher's exact test *P*-value.

Table 3 Comparison of results for previously reported mitochondrial SNPs and haplogroups among the identified studies

										Power (%)		
Study	*Associated SNP/haplogroup*	F_{CA}	F_{CO}	N_{CA}	N_{CO}	P-*value*	N_{SNPs}	N_{HG}	N_{TESTS}	*OR = 1.5*	*OR = 1.75*	*OR = 2*
Ray *et al.*[1]	T6221C	0.10	0.19	132	135	0.02	102	—	9	22.6	44.5	61.7
	T7389C	0.18	0.27	132	135	0.03	102	—	9	28.8	52.2	73.2
Booker *et al.*[2]	U	0.17	0.09	221	246	0.02	—	10	10	11.9	27.9	43.8
Canter *et al.*[3]	U	0.27	0.12	71	128	0.01	—	1	1	21.2	35.2	52.7
Álvarez-Cubero *et al.*[4]	U	0.16	0.15	239	150	0.66	125	10	43	14.5	27.7	38.2
Kim *et al.*[5]	U	0	0	139	122	—	—	22	31	—	—	—
Mueller *et al.*[6]	U	0.16	0.17	304	278	0.80	219	10	55	21.4	39.8	61.3
Wang *et al.*[7]	U (U1 + U2)	0.17	0.18	908	490	—	400	11	>2200	42.9	79.5	95.7
	U1	0.02	0.02			0.99				—	—	—
	U2	0.15	0.16			0.69				—	—	—
Present study	U	0.24	0.24	620	616	0.90	15	15	30	45.9	83.1	98.7

Abbreviations: F_{CA}, allele/haplogroup frequency in cases; F_{CO}, allele/haplogroup frequency in controls; N_{CA}, number of cases; N_{CO}, number of controls; N_{SNPs}, number of evaluated SNPs; N_{HG}, number of evaluated haplogroups; N_{TESTS}, number of performed tests; SNP, single-nucleotide polymorphism.

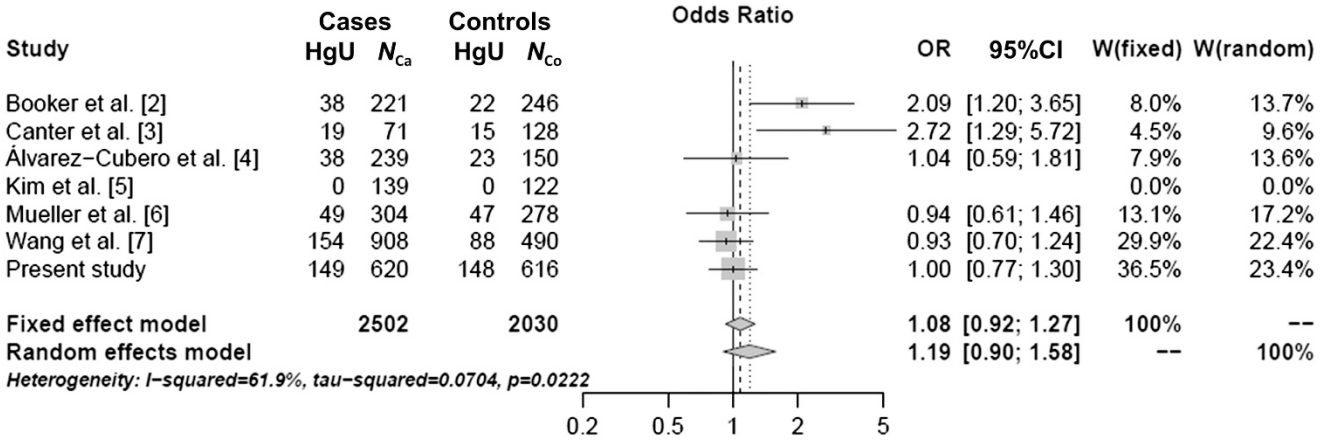

Figure 2 Meta-analysis carried out on haplogroup U using available data. HgU, sample size of haplogroup U individuals; N_{CA}, sample size of cases; N_{CO}, sample size of controls; W, weight of individual studies (in fixed- and random-effects models).

suggesting that haplogroup U is not associated with prostate cancer (at least considering a moderate risk increase above 1.75). We have also commented on some statistical/methodological issues that could explain spurious positive findings in the literature on prostate cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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