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Factors associated with oxidative stress and cancer risk in the Breast and Prostate Cancer Cohort Consortium (BPC3)

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Abstract

Both endogenous factors (genomic variations) and exogenous factors (environmental exposures, lifestyle) impact the balance of reactive oxygen species (ROS). Variants of the *ND3* (rs2853826; G10398A) gene of the mitochondrial genome, manganese superoxide dismutase (*MnSOD*; rs4880 Val16Ala) and glutathione peroxidase (*GPX-1*; rs1050450 Pro198Leu) are purported to have functional effects on regulation of ROS balance. In this study, we examined associations of breast and prostate cancer risk and survival with these variants, and interactions between rs4880 - rs1050450 and alcohol consumption - rs2853826. Nested case-control studies were conducted in the Breast and Prostate Cancer Cohort Consortium (BPC3), consisting of nine cohorts. The analyses included over 10726 post-menopausal breast and 7532 prostate cancer cases with matched controls. Logistic regression models were used to evaluate associations with risk, and proportional hazard models were used for survival outcomes. We did not observe significant interactions between polymorphisms in *MnSOD* and *GPX-1*, or between mitochondrial polymorphisms and alcohol intake and risk of either breast (p-interaction of 0.34 and 0.98 respectively) or prostate cancer (p-interaction of 0.49 and 0.50 respectively). We observed a weak inverse association between prostate cancer risk and *GPX-1* Leu198Leu carriers (OR 0.87, 95% CI 0.79 – 0.97, p = 0.01). Overall survival among women with breast cancer was inversely associated with G10398 carriers who consumed alcohol (HR 0.66 95% CI 0.49 – 0.88). Given the high power in our study, it is unlikely that interactions tested have more than moderate effects on breast or prostate cancer risk. Observed associations need both further epidemiological and biological confirmation.

Keywords

MnSOD; *GPX-1*; mitochondria; alcohol; breast; prostate

Introduction

Reactive oxygen species (ROS) are naturally occurring chemical entities derived from the metabolism of a number of compounds, in addition to being produced during oxidative respiration in the mitochondria. ROS are not only cytotoxic but are also mutagenic. Elevated levels of ROS and down regulation of ROS scavengers and/or antioxidant enzymes, which can lead to oxidative stress, are associated with a number of human diseases including various cancers [1, 2]. Oxidative damage to DNA can lead to changes such as single base modifications, gene duplications and the activation of oncogenes, and may be involved in the initiation of cancer.

Manganese superoxide dismutase and glutathione peroxidase are antioxidant enzymes, coded by the *MnSOD* and *GPX-1* genes respectively. The toxic superoxide anion ($O_2^{\cdot-}$) is a

naturally occurring by-product of the mitochondrial electron transport chain. Manganese superoxide dismutase acts as the first protective barrier against superoxide anion by metabolizing it into hydrogen peroxide. Hydrogen peroxide is further detoxified by *GPX-1*, a selenium-containing protein acting as a detoxifier of hydrogen products. These two enzymes contribute to regulate ROS exposures in the cell, and to prevent oxidative stress.

Various exogenous agents are known to affect ROS balance. Ozone and cigarette smoke [3], absorption of heavy metal particles [4], high dietary fat intake [5], and alcohol consumption [6, 7, 8] are environmental and lifestyle factors contributing to increased ROS production. Endogenous factors, and in particular genomic variations, may also play a key role in the regulation of ROS balance. Variants in genes involved in ROS regulation (antioxidant enzymes, ROS scavengers) or production (mitochondrial genes in charge of the respiratory chain) have been shown to alter their function and efficiency [9, 10].

For complex diseases like breast or prostate cancer, several low penetrance polymorphisms could have a synergistic effect leading to higher cancer risk [11]. Both breast [12, 13] and prostate [14, 15] cancer are reported to be linked to oxidative stress. Studies examining this hypothesis in specific candidate genes have been conducted in the Nurses' Health Study [16, 17, 18] with particular focus on gene-by-gene and gene-by-environment interactions related to ROS exposure.

The underlying hypothesis is that variations in the coding sequence leading to amino acid changes in the synthesized enzymes alter their function and efficiency [19, 20, 21], contributing to a deregulation of the balance of ROS, ultimately leading to oxidative stress. Individuals homozygous for the Ala16 allele of MnSOD and Leu198 allele of GPX-1 were observed to have a 1.87 fold increase in breast cancer risk compared to Val16 and Pro198 carriers, with a p-value for interaction of 0.03 in the Nurses' Health Study [16]. Furthermore, recently Méplan et al showed an association between breast cancer risk and alternative allele of GPX-1 polymorphism Pro198Leu ($p=0.027$).[23]

A second investigation in the Nurses' Health Study explored the impact of alcohol consumption on breast cancer risk for individuals carrying the mitochondrial SNP rs2853826/A10398G in the *ND3* gene [17]. Variations in the mitochondrial genome could affect the processing and the efficiency of the mitochondrial electron transport chain, leading to an increase in ROS production, and thus be associated with an increased risk of breast cancer. We previously showed that the 10398G allele modifies the association between alcohol consumption and breast cancer risk, with an odds ratio of 1.52 for drinkers compared to non-drinkers. No association between alcohol consumption and BC risk was observed among women carrying the 10398A allele.

In this study, we aimed to further investigate these hypotheses in independent populations. In addition, because oxidative stress may be linked to both breast and prostate cancers, we extend our research to prostate cancer risk. We genotyped rs2853826, rs4880, and rs1050450 in the NCI Breast and Prostate Cancer Cohort Consortium (BPC3), a large international consortium combining resources of nine well-established cohort studies [22]. While a large number of subjects in these cohorts were included in genome wide association

scans (GWAS [24, 25]), these three polymorphisms were not well represented on the specific products used in previous analyses. As such, any attempt to detect gene-by-gene and gene-by-environment interactions in large-scale genotyping efforts would not be informative for these specific hypotheses.

Materials and Methods

BPC3

The National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3) has been described previously [22]. In brief, the consortium combines resources from nine well-established cohort studies: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC), American Cancer Society Cancer Prevention Study II (CPSII), the European Prospective Investigation into Cancer and Nutrition Cohort (EPIC - composed of cohorts from Denmark, France, Great Britain, Germany, Greece, Italy, the Netherlands, Spain, and Sweden), the Health Professionals Follow-up Study (HPFS), the Multiethnic Cohort (MEC), the Physicians' Health Study (PHS), the Nurses' Health Study (NHS), the Women's Health Study (WHS), and the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Each study was approved by local Institutional Review Boards, and informed consent was obtained from all subjects.

Each cohort has its own method of case ascertainment, exposure assessment, and matching criteria to health controls [22]. 13511 post-menopausal women diagnosed with BC, and 8490 men diagnosed with prostate cancer were included in our study to analyze the interaction between the two polymorphisms respectively in *MnSOD* and *GPX-1*. 10726 post-menopausal women diagnosed with BC, and 7532 men diagnosed with prostate cancer were included in analyses the interaction between mitochondrial polymorphism rs2853826/A10398G and alcohol consumption.

As described in Hendrickson et al [26], ER/PR status is known for around 60% to 80% of breast cancers cases participating in the BPC3. ER status, follows the general distribution of around 20% ER- and 80% ER+ breast cancer. Given that a large number of cancer cases were ascertained prior to routine use, HER2 status, as well as other biomarkers like EGFR expression are not available.

Genotyping—Three single nucleotide polymorphisms (SNPs) were genotyped: rs1050450 (*GPX-1*), rs4880 (*MnSOD*) and the mitochondrial SNP rs2853826. For one cohort (PLCO) the SNP rs8031 (*MnSOD*), which is in high linkage disequilibrium with rs4880 ($r^2 = 0.95$ based on Hapmap genotype frequencies) was genotyped to infer the genotype of rs4880. Genotyping was performed via TaqMan assays. rs4880 and rs1050450 were in Hardy-Weinberg Equilibrium among controls, stratified by cohort. 334 subjects were excluded due to poor genotyping. For each SNP and each cohort, call rates were greater than 0.91. Cohorts with less than 90% success for a given SNP were removed from analyses for that SNP.

Statistical analysis—Regression analyses were carried out in R. Logistic regressions were conducted for each type of cancer, and for each of the 2 interactions tested. All analyses were performed under a recessive genetic model testing homozygotes for Leu allele

vs. Pro allele carriers, as previously published [17, 16]. Adjustment factors for each analysis are summarized in Table 1. Wald and Likelihood Ratio Test (LRT) p-values were computed to assess statistical interactions. Power calculations were performed using the Quanto software [26] (see Supplementary Table for details). Survival analyses among cases with respect to genotype and lifestyle factors were performed using proportional hazard models with the R-package *survival*. Heterogeneity tests were performed to evaluate the similarity of associations between cohorts, and random effects model estimates were retained when heterogeneity tests were significant ($p < 0.05$). A meta-analysis combining our data with published data concerning GPX-1 rs1050450 and PC was performed. 6 studies were initially selected to be included [28, 29, 30, 31, 32, 33]. However, Steinbrecher et al. [28] used data from the EPIC cohort, which is part of the BPC3. Thus we excluded this study, which might not be independent of our study. Results from Cheng et al. [30] were also excluded because information regarding homozygous and heterozygous Leu allele carriers was not presented. Dixon and Grubbs tests, from R package *outliers*, were used to assess whether outliers were present among all considered studies.

Results

For prostate cancer, we found an inverse association among homozygotes for the variant allele at rs1050450 of *GPX-1* (OR 0.87, 95% [0.79 -0.97], Tab. 1). Figure 1 presents results of a meta-analysis pooling our study of rs1050450 and prostate cancer with other published studies [29, 31, 32, 33]. We observed heterogeneity between studies ($p = 0.0033$), and random effects model estimation of global odds-ratio and confidence interval is 1.19 [0.79 - 1.80]. The Dixon and Grubbs tests identified the study of Kucukgergin et al. [32] as an outlier, and meta-analysis after exclusion of this study showed no between-study heterogeneity, with a fixed effects model odds-ratio of 0.90 (95% Confidence Interval 0.81 - 0.99). No interaction was detected between alcohol consumption and the *ND3* A10398G polymorphism (p -interaction = 0.50) with respect to prostate cancer risk or survival.

For breast cancer, we did not observe associations for the interaction between rs4880 Val16Ala in *MnSOD* and rs1050450 Pro198Leu in *GPX-1* (Table 1). Our study had greater than 95% power to detect an odds ratio of 1.87 as found in previous studies, at an α of 0.05, for an interaction between the recessive model for two polymorphisms where neither polymorphism alone is associated with risk. Neither of the 2 variants tested had an independent association with breast cancer risk. Carriers of both variant alleles were equally likely to develop breast cancer compared to reference allele carriers (OR = 1.03, 95% CI [0.97 -1.09]). No statistical interaction was detected between rs1050540 and rs4880 on breast cancer risk (p -interaction = 0.34). No difference in association between alcohol consumption and breast cancer risk was observed among carriers of the G or A alleles of the *ND3* A10398G polymorphism (p -interaction = 0.98). All results for breast cancer were similar when the Nurses' Health Study and Women's Health Study were removed (results not shown). Survival curves (Figure 2 and Table 2) are not statistically different between groups defined by genotypes for all analyses except for the mitochondrial A10398G genotype and alcohol consumption status among breast cancer patients. We observed a substantial difference between overall survival curves between different groups of genotype

and alcohol intake (Figure 2a and 2b). This effect is not present for breast cancer-specific survival (Figure 2c).

Discussion

The objective of the present study was to further evaluate associations of genomic variations involved in the alteration of reactive oxygen species balance with respect to breast and prostate cancer risk and survival. First, we focused on variations occurring in *MnSOD* and *GPX-1*, two genes encoding antioxidant enzymes that protect cellular DNA from oxidative damage. Carriers of both variant alleles for rs4880 Val16Ala in *MnSOD* and rs1050450 Pro198Leu in *GPX-1* did not have a change in risk of developing breast or prostate cancer.

Interestingly, we observed a weak inverse association between being homozygous Leu198Leu for rs1050450 (*GPX-1*) and risk of prostate cancer. This polymorphism has not been represented on previous GWAS products, and was only recently genotyped in the HapMap or in the 1000 Genomes project, so it is possible that this putative association was not detectable in prior GWAS, and inconsistent results were found for this association in candidate analyses. Although some studies observed a trend towards an inverse association between rs1050450 and prostate cancer for carriers of alternative allele of rs1050450 [28], others found an absence of association [33, 29, 34] or in an increase in risk for carriers of alternative alleles [32]. Our study is the first that shows an inverse association with a $p < 0.05$, but also the first having statistical power to detect a weak association, and the results of the meta-analysis reinforced our observations.

With respect to breast cancer, we observed no interaction between mitochondrial SNP rs2853826 and alcohol consumption. However, women who carry the G10398 allele and consume alcohol had longer survival both in terms of overall and disease-specific survival. Conversely, women who carry the A10398 allele and consume alcohol had reduced survival as compared to those who carry the same allele and do not consume alcohol. One hypothesized explanation for these results is the inverse association between moderate alcohol consumption and cardiovascular disease. As the majority of women in this study are post-menopausal (72%), and follow-up time is long (on average 8 years after diagnosis), there is potential for heart disease, independent of breast cancer status, to be a competing outcome. Furthermore, a number of adjuvant breast cancer therapies such as bevacizumab, taxane-anthracycline, and trastuzumab are associated with cardiovascular complications during BC treatment. Given the need to simplify our definition of alcohol consumption in order to combine data across participating cohorts, we have used a crude ever/never categorization. Further clarification of this putative association by more careful classification of alcohol consumption, particularly by type and quantity, may shed further light on these results. Additionally, information on treatments, unavailable for the vast majority of our study participants, will provide additional clarification in survival analyses.

Conclusion

In conclusion, we did not observe interactions between rs4880 in *MnSOD* and rs1050450 in *GPX-1* or rs2853826 and alcohol consumption and risk of breast or prostate cancer.

However we did observe a putative inverse association between rs1050450 in *GPX-1* and prostate cancer risk, and a novel interaction between alcohol consumption and rs2853826 in the mitochondrial *NAD3* gene on breast cancer survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BC	breast cancer
PC	prostate cancer
SNP	Single Nucleotide Polymorphism
OR	odds ratio
CI	confidence interval
GPX-1	glutathione peroxidase 1
MnSOD	manganese superoxide dismutase
ROS	reactive oxygen species

(a) *Top*. Forest-plots of odds-ratios with 95% confidence intervals for GPX-1 polymorphism and risk of prostate cancer Leu198Leu vs. Pro carriers (Ref.)

(b) *Bottom*. Forest-plots of odds-ratios with 95% confidence intervals for GPX-1 polymorphism and risk of prostate cancer - Leu198Leu vs. Pro carriers (Ref.) - after exclusion of Kucukgergin et al. [32]

Figure 1.

Meta-analysis pooling already published studies with our study in the BPC3, with and without study from Kucukgergin et al. [30]

Figure 2.
Survival plot (BC) with respect to genotype and alcohol consumption class

Table 1

Associations with breast and prostate cancer

	Genotype	Cases (%)	Controls (%)	OR (95% CI)
Interaction between GPX-1 Pro198Leu and MnSOD Val16Ala and breast cancer risk ¹	Pro198 carrier and Val16 carrier	3215 (66.3)	3685 (65.9)	1 (Ref.)
	Pro198 carrier and Ala16Ala	1134 (23.4)	1326 (23.7)	1.00 (0.96 – 1.03)
	Leu198Leu and Val16 carrier	371 (7.6)	442 (7.9)	1.00 (0.97 – 1.02)
	Leu198Leu and Ala16Ala	132 (2.7)	139 (2.5)	1.03 (0.97 – 1.09)
Interaction between mitochondrial A10398G and alcohol consumption on breast cancer risk ²	A10398, non-Drinkers	1114 (33.7)	1443 (36.2)	1 (Ref.)
	A10398, Drinkers	1507 (45.6)	1732 (43.5)	1.13 (1.02 – 1.26)
	G10398, non-Drinkers	294 (8.9)	372 (9.3)	1.03 (0.87 – 1.23)
	G10398, Drinkers	391 (11.8)	436 (10.9)	1.16 (0.99 – 1.36)
Association between GPX-1 Pro198Leu and prostate cancer risk ³	Pro198 Carrier	6688 (89.4)	6510 (88.2)	1 (Ref.)
	Leu198Leu	792 (10.6)	867 (11.8)	0.87 (0.79 – 0.97)
Interaction between GPX-1 Pro198Leu and MnSOD Val16Ala and prostate cancer risk ⁴	Pro198 carrier & Val16 carrier	4223 (66.2)	4230 (66.3)	1 (Ref.)
	Pro198 carrier & Ala16Ala	1473 (23.1)	1396 (21.9)	1.06 (0.97 – 1.15)
	Leu198Leu & Val16 carrier	507 (7.9)	573 (89)	0.88 (0.76 – 0.98)
	Leu198Leu & Ala16Ala	176 (2.8)	208 (3.3)	0.84 (0.67 – 1.02)
Interaction between mitochondrial A10398G and alcohol consumption on prostate cancer risk ⁵	A10398, non-Drinkers	389 (10.6)	429 (11.1)	1 (Ref.)
	A10398, Drinkers	2448 (66.7)	2596 (67.2)	1.15 (0.99 – 1.33)
	G10398, non-Drinkers	135 (3.7)	131 (3.4)	1.12 (0.85 – 1.48)
	G10398, Drinkers	698 (19.0)	706 (18.3)	1.16 (0.97 – 1.38)

¹ P-interaction = 0.34. Data restricted to post-menopausal women. Unconditional logistic regression controlled for age at blood draw, age at menarche, age at menopause, body mass index, family history of breast cancer, and cohort

² P-interaction = 0.98. Data restricted to post-menopausal women. Unconditional logistic regression controlled for age at blood draw, age at menarche, age at menopause, body mass index, family history of breast cancer, and cohort

³ Unconditional logistic regression controlled for age at diagnosis, alcohol consumption, and cohort

⁴ P-interaction = 0.44. Unconditional logistic regression controlled for age at diagnosis, alcohol consumption, and cohort

⁵ P-interaction = 0.50. Unconditional logistic regression controlled for age at diagnosis and cohort

Table 2**Survival analyses for breast and prostate cancer**

Cancer Type	Analysis	Category	Overall Survival		Specific Survival	
			Odd-Ratios and 95% Confidence Interval	Log-Rank Test Pvalue	Odd-Ratios and 95% Confidence Interval	Log-Rank Test Pvalue
BREAST	2 SNPs GP $\times$ 1/MnSOD *	Pro198 carrier - Val16 carrier	1 (Ref.)	0.747	1 (Ref.)	0.508
		Pro198 carrier - Ala16Ala	0.93 (0.79 - 1.10)		1.09 (0.87 - 1.36)	
		Leu198Leu - Val16 carrier	0.93 (0.72 - 1.21)		0.85 (0.57 - 1.26)	
		Leu198Leu - Ala16Ala	0.85 (0.55 - 1.32)		0.74 (0.38 - 1.43)	
	Alcohol $\times$ mtSNP A10398G *	A10398 - Non-Drinkers	1 (Ref.)	0.029	1 (Ref.)	0.103
		A10398 - Drinkers	0.9 (0.76 - 1.07)		1.24 (0.97 - 1.57)	
		G10398 - Non-Drinkers	1.03 (0.77 - 1.36)		0.95 (0.62 - 1.44)	
		G10398 - Drinkers	0.66 (0.49 - 0.88)		0.84 (0.57 - 1.26)	
	Alcohol effect only *	Non-Drinkers	1 (Ref.)	0.003	1 (Ref.)	0.104
		Drinkers	0.84 (0.74 - 0.94)		1.15 (0.97 - 1.36)	
PROSTATE	2 SNPs GP $\times$ 1/MnSOD **	Pro198 carrier - Val16 carrier	1 (Ref.)	0.855	1 (Ref.)	0.356
		Pro198 carrier - Ala16Ala	1.00 (0.89 - 1.12)		0.93 (0.77 - 1.14)	
		Leu198Leu - Val16 carrier	1.00 (0.84 - 1.18)		0.75 (0.54 - 1.04)	
		Leu198Leu - Ala16Ala	1.13 (0.86 - 1.49)		1.05 (0.64 - 1.7)	
	Alcohol $\times$ mtSNP A10398G **	A10398 - Non-Drinkers	1 (Ref.)	0.558	1 (Ref.)	0.148
		A10398 - Drinkers	1.07 (0.87 - 1.32)		1.17 (0.82-1.67)	
		G10398 - Non-Drinkers	0.85 (0.57 -1.27)		0.49 (0.21-1.16)	
		G10398 - Drinkers	1.09 (0.86 - 1.38)		1.13 (0.75-1.69)	

* Adjusted for Cohort and Age at Breast Cancer diagnosis

** Adjusted for Cohort and Age at Prostate Cancer diagnosis