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ORIGINAL ARTICLE

Mitochondrial DNA variant interactions modify breast cancer risk

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Abstract Interactions between mitochondrial deoxyribonucleic acid (mtDNA) variants and the risk of developing breast cancer were investigated using DNA samples collected from non-Jewish European American breast cancer patients and ethnically age-matched female controls. Logistic regression was used to evaluate two-way interactions between 17 mtDNA variants. To control for multiple testing, empirical *P* values were calculated using permutation. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to measure the contribution of variants in modifying the risk of developing breast cancer. A highly significant interaction was identified between variants 12308G and 10398G (empirical *P* value = 0.0028), with results suggesting these variants increase the risk of a woman developing breast cancer (OR = 3.03; 95% CI 1.53–6.11). Nominal significant *P* values were also observed for interactions between mtDNA variants 709A and 16189C; 4216C and 10398G; 4216C and 16189C; 10398G and 16159C; 13368A and 16189C; and 14766T and 16519C. However, after adjusting for multiple testing, the *P* values did not remain significant. Although it is important to elucidate the main effect of mtDNA variants on the risk of developing breast cancer, understanding gene × gene interactions will give a greater knowledge of disease etiology and aid in interpreting a woman's risk of developing breast cancer.

Keywords Mitochondrial DNA · Breast cancer · Interaction

Introduction

Breast cancer is the most diagnosed cancer in women and has the second highest mortality rate among cancers in women. Both environmental (e.g., hormone replacement therapy, age at menopause, obesity, etc.) and genetic factors influence a woman's risk of developing breast cancer. Established high-risk cancer genes *BRCA1*, *BRCA2*, *PTEN*, *TP53, LKB1/STK11*, *CDH1* and low-to-moderate risk cancer genes *CHEK2*, *TGFβ1*, *CASP8*, *ATM* account for 25% of familial and 5–10% of breast cancer cases within the general population (Antoniou and Easton 2006; Levy-Lahad and Friedman 2007).

Mitochondria contain their own genome, mitochondrial deoxyribonucleic acid (mtDNA), which is a 16.5-kb circular double-stranded DNA (dsDNA) molecule containing 37 genes. MtDNA is multicopy and maternally inherited. The most prominent roles of mitochondria are production of adenosine triphosphate (ATP) and regulation of cellular metabolism. Mutations within the mtDNA are known to cause various disease phenotypes, such as sensorineural hearing impairment (Forli et al. 2007; Kokotas et al. 2007), Leber's hereditary optic neuropathy (Yen et al. 2006), mitochondrial myopathy (Wong 2007), and mitochondrial encephalopathy, lactacidosis, and stroke-like episodes syndrome (MELAS) (Filosto et al. 2007; Finsterer 2007). Recently, mtDNA variants have been implicated in various

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age-related disorders and complex diseases such as Parkinson's disease (Mortiboys et al. 2007; Onyango 2008), Alzheimer's disease (Carrieri et al. 2001), and several cancers (Petros et al. 2005). Due to its haploid nature and lack of recombination, it is possible to classify individuals through specific mtDNA variants that make up haplogroups. These haplogroups have been shown to possess both geographical-region- and ethnic-specific differences in prevalence. For example, Sub-Saharan Africans mainly carry the L haplogroup, whereas haplogroups H, I, J, and K are predominant in the European populations, with Ashkenazi Jews more frequently carrying the K haplogroup compared with non-Jewish Europeans (Torroni et al. 1996). This study investigates the role of interaction between mtDNA variants on the risk of developing breast cancer.

Recent studies provide evidence that germline mtDNA variants T3197C, G13708A, G9055GA, T16519C, and A10398G and/or functional variants in linkage disequilibrium (LD) with these variants modify a woman's risk of developing breast cancer (Canter et al. 2005; Bai et al. 2007). In African American women, it was shown that 10398A increases the risk of developing breast cancer (Canter et al. 2005), whereas in European American women, 10398G increases the risk of developing breast cancer (Bai et al. 2007). Additionally, there is evidence that mtDNA variants T3197C, G13708A, G9055A, and T16519C modify breast cancer risk in European American women (Bai et al. 2007). Although it is important to understand the main effects of mtDNA variants on breast cancer risk, knowledge of how mtDNA variants interact gives greater insight into breast cancer etiology and aids in risk assessment. We investigated interactions between mtDNA variants in a case–control study of non-Jewish European American women. Seven significant mtDNA interactions were detected, with one interaction (12308G:10398G; empirical P value = 0.0028) remaining significant after adjusting for multiple testing.

Materials and methods

Sixty-nine mtDNA variants were genotyped in DNA samples from 156 non-Jewish European American breast cancer patients and 260 ethnically age matched female controls. Of these 69 mtDNA variants, 19 were polymorphic and had minor allele frequencies of $\geq$0.05. Two of these variants, A4917G and A15607G, were correlated ($r^2 > 0.8$) with mtDNA variant G13368A and were not included in the logistic regression analysis. The following 17 mtDNA variants were evaluated for all possible two-way interactions: G709A, G1719A, T3197C, T4216C, G5460A, C7028T, G8251A, G9055A, A10398G, A12308G, G13368A, G13708A, C14766T, A15924G, C16069T, T16189C, and T16519C. Additional details on subject ascertainment, mtDNA genotyping, and initial analysis can be found in Bai et al. (2007).

Two-way interactions were evaluated using logistic regression by modeling the main effects of two mtDNA variants and the corresponding interaction term

$$\log \frac{\theta(\mathbf{x})}{1-\theta(\mathbf{x})} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 (x_1 * x_2),$$
$$\text{where } \theta(\mathbf{x}) = P(\text{Case}|\mathbf{x}). \quad (1)$$

A total of 136 logistic regression models were evaluated. The common allele within this European American sample was used as the reference allele. Significance was evaluated for the interaction term (H_0: $\beta_3 = 0$; the interaction had no effect, versus $H_1 : \beta_3 \neq 0$; the interaction had an effect) using the Wald test, as well as for the complete model (H_0:$\beta_1 = \beta_2 = \beta_3 = 0$; the mtDNA variants and their interaction had no effect, versus $H_1 : \beta_i \neq 0$ for an i = 1, 2, 3; one or more of the mtDNA variants and/or their interaction had an effect) using the likelihood ratio test. Due to the large number of tests carried out, the family-wise error rate (FWER) was controlled by calculating empirical P values using 10,000 permutations. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for the mtDNA variant main effects and pair-wise interactions.

Results

Table 1 summarizes the allelic frequencies for the 17 mtDNA variants found in the DNA samples from cases and controls. Logistic regression analyses revealed seven nominally significant interactions among the variants: 709A and 16189C; 4216C and 10398G; 4216C and 16189C; 12308G and 10398G; 10398G and 16519C; 13368A and 16189C; and 14766T and 16519C (Table 2). After controlling the FWER, the only interaction term that remained statistically significant was between 10398G and 12308G (permuted P value 0.0028). Individual analyses of these two variants using logistic regression analysis (Table 3) revealed A10398G had a statistically significant main effect (logistic regression $P = 0.011$) and increased a woman's risk of developing breast cancer (OR = $e^{0.588} = 1.8$; 95% CI 1.14–2.81) (Table 3). The main effect of variant A12308G was not significant (logistic regression $P = 0.84$) (Table 3). Using the complete model with an interaction term (Eq. 1), the logistic regression results suggest women who have both the 10398G and 12308G variants had an increased risk of developing breast cancer (OR = $e^{-0.771-0.006+1.887} = 3.03$; 95% CI 1.53–6.11) (Table 4). Whereas women who carry the 10398A variant and the 12308G variant had a decreased risk of developing breast cancer (OR = $e^{-0.771} = 0.46$;

Table 1 Summary of the 17 mitochondrial deoxyribonucleic acid (mtDNA) variants included in the analysis

SNPs	Cases ($n = 156$)		Controls ($n = 260$)		Odds ratio
	Positive	%	Positive	%	
G709A	20	12.82	25	9.62	1.39
G1719A	17	10.90	18	6.92	1.64
T3197C	6	3.85	31	11.92	0.31
T3394C	3	1.92	4	1.54	1.30
T4216C	26	16.67	52	20.00	0.81
G5460A	4	2.56	18	6.92	0.38
C7028T	91	58.33	149	57.31	1.04
G8251A	16	10.26	16	6.15	1.74
G9055A	29	18.59	18	6.92	3.03
A10398G	50	32.05	54	20.77	1.79
A12308G	41	26.28	66	25.38	1.05
G13708A	12	7.69	40	15.38	0.47
C14766T	84	53.85	137	52.69	1.04
A15924G	15	9.62	16	6.15	1.62
C16069T	10	6.41	30	11.54	0.54
T16189C	21	13.46	35	13.46	1.01
T16519C	122	78.21	167	64.23	1.98

Displayed in the table are the mtDNA variant name, the number of variants present in cases and controls, and the percentage of cases and controls with the variant

SNPs single nucleotide polymorphisms

Table 2 Significant two-way interactions

Interaction	Logistic interaction P value	Adjusted interaction P value	Logistic regression global P value
709A:16189C	0.0081	0.1102	0.0235
4216C:10398G	0.0079	0.1070	0.0009
4216C:16189C	0.0150	0.2190	0.0752
12308G:10398G	0.0004	0.0028	0.0002
10398G:16519C	0.0221	0.3289	0.0001
13368A:16189C	0.0099	0.1392	0.0440
14766T:16519C	0.0158	0.2304	0.0016

Displayed in the table are the names of the mtDNA variants, nominal interaction P values ($H_0 : \beta_3 = 0$; $H_1 : \beta_3 \neq 0$), interaction P values adjusted for the family-wise error rate (FWER) and nominal global P values ($H_0 : \beta_1 = \beta_2 = \beta_3 = 0$; $H_1 : \beta_i \neq 0$ for one or more $i = 1, 2, 3$) obtained from analysis of deviance

95% CI 0.24–0.88) (Table 4). Additionally, in the presence of variant 12308A, variant 10398G had no effect on breast cancer risk (OR $= e^{-0.006} = 0.99$, $P = 0.98$; 95% CI 0.56–1.77) (Table 4).

Variants 10398A and 12308A define the T haplogroup along with variants 4216C, 7028T, 13368A, and 15607G. Variants 10398A and 12308G define the U haplogroup along with the variant 9055G. Although belonging to the T haplogroup does not influence a woman's risk of developing breast cancer, membership in the U haplogroup has been shown to greatly reduce a woman's risk of developing breast cancer [OR = 0.38; P value = 0.03 (adjusted for multiple testing)] (Bai et al. 2007). In this analysis, it can be seen that regardless of G9055A status, a woman carrying the mtDNA variants 12308G and 10398A has a reduced risk of developing breast cancer (OR $= e^{-0.771} = 0.46$; 95% CI 0.24–0.88) (Table 4).

Discussion

The 10398A variant has previously been implicated in increasing the risk of developing both breast and prostate cancer in African Americans (Canter et al. 2005; Mims et al. 2006) and breast and esophageal cancer in Indians (Darvishi et al. 2007), whereas the 10398G variant increases the risk of developing breast cancer in European American women (Bai et al. 2007). Two independent studies could not replicate the findings that the 10398A variant increases the risk of developing breast cancer in African American women (Setiawan et al. 2008) or that the 10398G variant increases the risk of developing breast cancer in women from Spain and the Canary Islands (Mosquera-Miguel et al. 2008).

One of the interactions, 4216C and 10398G, observed in this study although not statistically significant after controlling the FWER (Table 2), was previously reported in the Canter et al. (2005) case–control study of African American breast cancer patients (Verma et al. 2007). In the Canter study, a significant main effect was observed for 10398A, which increased a woman's risk of developing breast cancer (OR = 1.6), whereas no statistically significant main effect was observed for T4216C and a synergistic interaction was observed between 4216C and 10398A, where the presence of both variants increased a woman's risk of developing breast cancer (OR = 3.1) (Verma et al. 2007). In this case-control study of European Americans, when each variant was analyzed independently, an increased risk was observed for 10398G (OR $= e^{0.588} = 1.8$; $P = 0.01$) (Table 3), and there was also no statistically significant main effect observed for T4216C variant ($P = 0.39$) (data not shown). This study observed a nominally significant interaction between 4216C and 10398G ($P = 0.008$), which did not remain significant after controlling the FWER ($P = 0.11$) (Table 2). Although women having the 4216T and 10398G variants had an increase in developing breast cancer (OR $= e^{1.106} = 3.02$; 95% CI 1.72–5.32), women with the 4216C and 10398G variants had a decreased risk of developing breast cancer (OR $= e^{0.191+1.106-1.492} = 0.82$;

Table 3 Coefficient estimates for mitochondrial deoxyribonucleic acid (mtDNA) variants A10398G and A12308G

Parameters	Variant(s)	Coefficient estimate (standard error)			Parameter *P* value	Global *P* value
		A12308G	*A10398G*	Interaction		
β_1	12308G[a]	0.047 (0.231)	–	–	0.839	0.841
β_2	10398G[b]	–	0.588 (0.230)	–	0.011	0.011
$\beta_1 + \beta_2$	12308G and 10398G[c]	−0.072 (0.238)	0.602 (0.235)	–	0.762, 0.010[e]	0.037
$\beta_1 + \beta_2 + \beta_3$	12308G:10398G[d]	−0.771 (0.329)	−0.006 (0.294)	1.887 (0.53)	0.0004[f]	0.0005

Analyses were carried out including only A12308G in the model; including only A10398G in the model; including A12308G and A10398G in the model without an interaction term; and including A12308G, A10398G with an interaction term in the model. Displayed in the table are the parameters, names of variants included in the logistic regression model, coefficient estimates and their standard error, parameter *P* values (Wald test), and global *P* values (logistic regression) obtained from analysis of deviance

[a] Only A12308G included in the logistic regression model

[b] Only A10398G included in the logistic regression model

[c] Variants A10398G and A12308G included in the model, no interaction term included in the logistic regression model

[d] Variants A10398G and A12308G included in model with an interaction term included in the logistic regression model

[e] *P* values for β_1 and β_2, respectively

[f] *P* value for β_3 only

Table 4 Summary of results from the logistic regression for mitochondrial deoxyribonucleic acid (mtDNA) variants A10398G and A12308G

Parameter	Variant	$\hat{\beta}_i$ (coef. est.)	Standard error	*Z* value	Parameter *P* value
β_0	–	−0.052	0.132	−3.964	7.39e-05
β_1	12308G	−0.771	0.329	−2.343	0.019
β_2	10398G	−0.006	0.294	−0.022	0.983
β_3	12308G:10398G	1.887	0.530	3.559	0.0004

Displayed in the table are the parameters, names of variants, estimates of the β coefficients, estimates of the standard error, *Z* values, and parameter *P* values

Table 5 Summary of results from the logistic regression for mitochondrial deoxyribonucleic acid (mtDNA) variants T4216C and A10398G

Parameter	Variant	$\hat{\beta}_i$ (coefficient estimate)	Standard error	*Z* value	Parameter *P* value
β_0	–	−0.688	0.128	−5.381	7.41e-08
β_1	4216C	0.191	0.362	0.528	0.598
β_2	10398G	1.106	0.288	3.849	0.0001
β_3	4216C:10398G	−1.492	0.561	−2.657	0.008

Shown in the table are parameters, variant names, β coefficient estimates, standard error estimates, *Z* values, and parameter *P* values

95% CI 0.39–1.67) (Table 5). For variant A10398G, the Canter study (2005) used 10398G as the reference group, because it is the common allele in African Americans. In European Americans, 10398A is the common allele and was used as the reference group in this study. When the same reference group (10398G) was used as in the Canter study in the logistic regression model, women who had the 4216C and 10398A variants had a decreased risk of developing breast cancer ($OR = e^{-1.11-1.30+1.492} = 0.40$; 95% CI 0.17–0.93) (data not shown). The differences in direction of the effect between the Canter and this study suggest the functional variants that increase a woman's risk of developing breast cancer are on different haplotypes in African American and European American women.

This study presents strong evidence that genes within the mitochondria interact to increase the risk of breast cancer. It is demonstrated that mtDNA variants A12308G and A10398G located within the *MT-TL2* and *MT-ND3* genes, respectively, interact, affecting a woman's risk of developing breast cancer. In particular, mtDNA variants 10398G and 12308G interact synergistically, increasing a woman's risk of developing breast cancer.

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