Is Mitochondrial DNA Variation Associated with Sporadic Breast Cancer Risk?

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To the Editor:

In the May 15, 2007 issue of *Cancer Research*, Bai et al. (1) claimed that individuals carrying haplogroup K mitochondrial DNA (mtDNA) lineages are at significantly increased risk of developing breast cancer, whereas those bearing haplogroup U lineages have a significantly decreased risk. However, this study has a number of drawbacks. First, haplogroup K is phylogenetically nested within haplogroup U; consequently, diagnostic mutations in haplogroup U are necessarily also diagnostic in haplogroup K (in particular, A11467G, A12308G, and G12372A). Therefore, how can there be 29 cases belonging to haplogroup K but only 12 belonging to the broader group U? This is not trivial because these were the only two haplogroups found to be associated with breast cancer risk after correction for multiple testing. Second, the study does not investigate nor even mention the potential influence of population stratification in their sample of 156 European-American breast cancer patients and 260 controls, nor are their results replicated in an independent population. The door is therefore open to false-positive findings (type I error).

We have collected breast cancer patients and ethnicity-matched controls from two different Spanish locations. One sample consisted of 464 cases and 453 controls from continental Spain (2), whereas the second, from Canary Islands, included 302 cases and 295 controls. The first sample has been tested for population stratification using a panel of neutral single nucleotide polymorphisms (SNP; ref 2). Our samples were genotyped for a set of 25 mtDNA SNPs, including all those with evidence of association based on unadjusted *P* values in (1) as well as the variant G10398A reported in (3, 4). We found no evidence of association for any of the variants after adjustment for multiple testing in either sample (Table 1). With the Spanish mainland sample alone, we had ~80% power to detect odds ratios (OR) as low as 2.00 for G9055A and 1.50 for A12308G (the two variants that define haplogroups K and U, respectively). In fact, OR estimates for the variants G9055A and A12308G were in the opposite direction to those reported in (1): 0.65 [95% confidence interval (95% CI), 0.36–1.18] and 0.87 (95% CI, 0.45–1.66) in Spanish mainland and Canary Island samples, respectively, for G9055A; and 0.74 (95% CI, 0.55–0.99) and 0.77 (95% CI, 0.52–1.14), respectively, for A12308G.

MtDNA variation is deeply structured in populations. These markers are therefore more susceptible to false-positive findings in association studies than autosomal SNPs (5). Phenotype alone is not sufficient to rule out the existence of different mtDNA ancestries in a population sample (this is especially true in countries such as the USA; ref. 6). In this regard, the positive SNP associations with breast cancer found by other authors (3, 4) are also questionable. Assessing population stratification and/or replication of positive associations using independent samples is therefore essential in all association studies, and even more so in those of MtDNA. We conclude that although it is possible that mtDNA variation is associated with breast cancer risk, this remains to be properly shown.

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Table 1. Summary of results for individual mtSNPs loci in two independent Spanish case control studies

HG (haplogroup)*	SNP variant †	Spanish mainland				
		Cases *N* (%)	Controls *N* (%)	*P*§	Adjusted *P* value‖	OR (95% CI)¶
H→H1	G3010A	107 (25%)	135 (32%)	0.047**	0.540	0.73 (0.44–1.00)
U→U5	T3197C	34 (7%)	22 (4%)	0.099	0.825	1.61 (0.89–2.93)
H6→H6a/H→H17	G3915A	8 (1%)	10 (2%)	0.642	1.000	0.78 (0.27–2.23)
H→H4	C3992T	7 (1%)	6 (1%)	1.000	1.000	1.15 (0.33–4.18)
R→JT	T4216C	81 (17%)	81 (17%)	0.931	1.000	0.98 (0.69–1.39)
H5→H5a	T4336C	11 (2%)	14 (3%)	0.548	1.000	0.76 (0.31–1.84)
N→I	A4529T	10 (2%)	7 (1%)	0.626	1.000	1.40 (0.48–4.38)
HV0a→V	G4580A	16 (3%)	20 (4%)	0.499	1.000	0.77 (0.37 1.60)
H2a→H2	A4769G	452 (97%)	447 (98%)	0.328	0.997	0.55 (0.17 1.64)
H→H7	A4793G	0 (0%)	0 (0%)	1.000	1.000	—
H→H3	T6776C	39 (8%)	42 (9%)	0.727	1.000	0.91 (0.56–1.47)
H→HV	C7028T	240 (51%)	225 (49%)	0.552	1.000	1.09 (0.83–1.42)
N2→W	G8994A	6 (1%)	6 (1%)	1.000	1.000	1.01 (0.27–3.80)
U→K	G9055A	22 (4%)	33 (7%)	0.163	0.941	0.65 (0.36–1.18)
JT→J/K→K1/N→I	A10398G	78 (16%)	82 (18%)	0.664	1.000	0.93 (0.65–1.32)
N→M	C10400T	2 (0%)	7 (1%)	0.105	0.842	0.28 (0.03–1.47)
JT→T	T10463C	46 (10%)	45 (10%)	1.000	1.000	1.01 (0.64–1.60)
N→L3*	T10873C	9 (1%)	12 (2%)	0.514	1.000	0.73 (0.27–1.90)
R0→R	G11719A	111 (39%)	161 (44%)	0.171	0.952	0.80 (0.57–1.11)
R→U	A12308G	80 (17%)	80 (17%)	0.931	1.000	0.98 (0.68–1.39)
R→N	C12705T	35 (8%)	29 (6%)	0.519	1.000	1.19 (0.69–2.06)
R→J	G13708A	34 (8%)	41 (9%)	0.717	1.000	0.90 (0.54–1.48)
N→X	A13966G	8 (1%)	4 (0%)	0.385	0.999	1.97 (0.53–9.01)
HV→R0	C14766T	198 (42%)	189 (41%)	0.738	1.000	1.05 (0.80–1.38)
—	T16519C	280 (63%)	310 (69%)	0.039**	0.478	0.74 (0.55–0.99)

*HG is a proxy for haplogroup status; note that some mutations are characteristic of different phylogenetic pathways. Only the most relevant ones are shown in the table; for instance, A12308G defines haplogroup U, and G9055A leads to subclade haplogroup K; T16519C is too mutationally unstable and does not identify any haplogroup *per se*.

†Mutations are referred to in accordance with the revised Cambridge reference sequence (rCRS).

‡*P* values as reported in (1).

§*P* value for Fisher's exact test.

‖Adjustment for multiple testing by permutation (N = 10.000).

¶OR for the variant allele with respect to rCRS; exact 95% CI.

**$P < 0.05$.

Table 1. Summary of results for individual mtSNPs loci in two independent Spanish case control studies (Cont'd)

Canary Islands					Adjusted *P* value (Bai's study)[‡]
Cases *N* (%)	Controls *N* (%)	*P*[§]	Adjusted *P* value[‖]	OR (95% CI)[¶]	
64 (21%)	45 (15%)	0.072	0.689	1.48 (0.95–2.31)	—
22 (7%)	8 (2%)	0.015**	0.178	2.72 (1.14–7.18)	0.0526
5 (1%)	5 (1%)	1.000	1.000	0.98 (0.22–4.29)	—
3 (1%)	2 (0%)	1.000	1.000	1.48 (0.17–17.9)	—
55 (18%)	62 (21%)	0.534	1.000	0.87 (0.57–1.33)	0.99
2 (0%)	2 (0%)	1.000	1.000	0.98 (0.07–13.6)	0.99
4 (1%)	0 (0%)	0.124	0.852	—	0.86
8 (2%)	2 (0%)	0.107	0.794	4.00 (0.79 38.9)	1
302 (100%)	295 (100%)	1.000	1.000	—	—
1 (0%)	1 (0%)	1.000	1.000	0.96 (0.01–75.8)	—
13 (4%)	7 (2%)	0.257	0.987	1.82 (0.66–5.46)	—
208 (68%)	217 (73%)	0.281	0.992	0.81 (0.56–1.17)	1
3 (1%)	5 (1%)	0.499	1.000	0.58 (0.09–3.00)	1
22 (7%)	24 (8%)	0.759	1.000	0.87 (0.45–1.66)	0.0057[¶]
75 (24%)	66 (22%)	0.439	1.000	1.17 (0.79–1.75)	0.19
6 (1%)	3 (1%)	0.505	1.000	1.97 (0.42–12.3)	—
34 (13%)	44 (18%)	0.137	0.888	0.67 (0.40–1.13)	—
33 (10%)	25 (8%)	0.335	0.996	1.32 (0.74–2.30)	—
186 (63%)	201 (71%)	0.054	0.518	0.70 (0.49–1.01)	—
88 (29%)	112 (37%)	0.019**	0.213	0.66 (0.46–0.94)	1
45 (14%)	35 (11%)	0.337	0.996	1.29 (0.78–2.14)	—
27 (9%)	18 (6%)	0.153	0.909	1.66 (0.84–3.37)	0.267
7 (2%)	4 (1%)	0.545	1.000	1.73 (0.43–8.12)	—
194 (64%)	209 (70%)	0.117	0.829	0.75 (0.53–1.08)	1
217 (72%)	221 (77%)	0.181	0.945	0.77 (0.52–1.14)	0.0366[¶]

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