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Rapid Communication

The MnSOD Val9Ala polymorphism, dietary antioxidant intake, risk and survival in ovarian cancer (Australia)

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Abstract

Objective. We assessed the MnSOD Val9Ala polymorphism and its interaction with dietary antioxidant intake in ovarian cancer risk and survival.
Methods. The MnSOD polymorphism was assessed in 543 ovarian cancer cases and 1130 controls. We used regression analysis to model the association between genotype and risk, case-only analyses to estimate risk modification by dietary variables, and proportional hazard models for survival analysis.
Results. We found no association between this polymorphism and ovarian cancer risk or survival, nor was there evidence of any interaction with dietary antioxidant intake.
Conclusion. The Val9Ala MnSOD polymorphism does not influence ovarian cancer risk or survival.

Keywords: MnSOD polymorphism; Ovarian cancer; Diet; Etiology; Survival

Introduction

Manganese superoxide dismutase (MnSOD) is a mitochondrial matrix enzyme that scavenges reactive oxygen species (ROS) and protects the cell against the insults of oxidative stress [1]. Transgenic animal studies demonstrating increased tumor incidence associated with reduced MnSOD activity, and diminished antioxidant enzyme levels in cancer cells and tumors, implicate MnSOD as a tumor suppressor gene [2,3]. One hypothesis for ovarian carcinogenesis is that rapid cell division of the ovarian epithelium, and the inflammatory processes associated with ovulation may lead to the production of ROS, resulting in oxidative damage to DNA [4]. The valine variant of the Valine–9Alanine polymorphism is the result of a T → C substitution at position −9 of the mitochondrial targeting sequence (MTS), the signal peptide necessary for transport of MnSOD from the cytosol into the mitochondrion. This variant is predicted to alter protein conformation, and is believed to compromise transport and localization of MnSOD, leaving the cell vulnerable to oxidative damage [5]. One hospital-based case–control study of 125 ovarian cancer cases and 193 controls reported a twofold increased risk among carriers of one or two alanine alleles compared to valine homozygotes [odds ratio (OR) 2.1; 95% confidence interval (CI) 1.1–4.0] [6]. We report the results of risk and survival analyses associated with the MnSOD Val9Ala polymorphism in a large Australian ovarian cancer case–control study, as well as case-only analyses to estimate possible risk modification by dietary antioxidant intake.

Materials and methods

Approval for this study was obtained from the ethics committees of the University of Queensland, University of Melbourne, Queensland Institute of Medical Research, Queensland Cancer Fund, New South Wales Cancer Council, Cancer Council Victoria, National Death Index and all metropolitan hospitals where patients were recruited.

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Table 1
Characteristics of ovarian cancer cases and controls

	Ovarian cancer cases, $N=543$	Twin controls (a), $N=300$	ABCFS controls (b), $N=830$
Age in years[a] (mean±SD)	57.4±13.4	50.9±13.9	42.0±10
Any live births (%)	77.2	87.1	73.0[b]
Age at menarche ≥12 (%)	86.7	85.7[b]	82.7[b]
Family history of ovarian cancer[c] (%)	2.3	[d]	[d]
Family history of breast cancer[c] (%)	10.0	[d]	6.6[b]
Any oral contraceptive use (%)	49.4	[d]	89.4

[a] Age at diagnosis for cases and age at interview for controls.
[b] No significant difference between cases and controls.
[c] Family history is any reported 1st-degree relative.
[d] Data not available.

We analyzed data from 543 women with epithelial ovarian cancer, with tumor histology as follows: 320 (59.0%) serous, 68 (12.5%) mucinous, 62 (11.4%) endometrioid, 32 (5.9%) clear cell carcinoma, 36 (6.6%) mixed cell, 25 (4.6 %) other histologies, and 1130 controls. Cases were ascertained from the Royal Brisbane Hospital, Queensland, and from gynecologic–oncology treatment centers in Australia as part of a population-based case–control study [7]. Controls consisted of two groups: (a) 300 unrelated adult female monozygotic twins (one per pair) from a random sample of 3348 twins recruited through the volunteer Australian Twin Registry [8,9] and selected to best match the date-of-birth distribution of ovarian cancer cases, and (b) 830 women without breast cancer who participated in the Australian Breast Cancer Family Study [10]. Selected characteristics of cases and controls are presented in Table 1 and have been previously described [8].

A total of 312 cases from the population-based study also self-completed a food frequency questionnaire (119 food items), modified to reflect Australian foods and portion sizes. Women were asked to report their usual intake over the year prior to the onset of symptoms they related to their diagnosis.

Information on diagnosis, disease stage (using the International Federation of Gynecologists and Obstetricians [FIGO] criteria), tumor histology, grade and treatment was abstracted retrospectively from the women's medical records and pathology reports or, for a subset of cases, from the Royal Brisbane Hospital Gynecology Oncology database. Full details have been reported previously [11]. Where possible, the cases were followed for mortality using personal identifiers which were linked to state cancer registry records, the Australian National Death Index (NDI) and the hospital Gynecology Oncology database. We had follow-up data for 447 cases (82% of all cases).

The MnSOD Val9Ala polymorphism was detected using the ABI Prism 7700 Sequence Detection System (SDS). An 84-bp polymerase chain reaction product was amplified using the primers GGCTGTGCTTTCTCGTCT and GGCTGTGCTTCTGCCTGGA, and fluorescently labeled probes 5′-6-carboxy-fluorescein (FAM)-CAGATACCCCAAAgCCGGAAGCC-6-carboxy-tetra-methyl-rhodamine (TAMRA)-3′ and 5′-tetrachloro-6 carboxy-fluorescein (TET)-CAGATACCCCAAAaCCGGAGCCA-TAMRA-3′ were used to detect the C and T alleles, respectively. The 20 μl PCR reaction volume included 30 ng pre-dried genomic sample DNA, and a final concentration of 1× *Taq*Man Universal PCR Master Mix (The Perkin-Elmer Corp., Foster City, CA, Perkin Elmer Catalogue No. 4304437), 900 nM each primer, 100 nM FMA-A probe, and 100 nM VIC-G probe. Reactions were amplified in the ABI 7700 SDS PCR machine for 2 min at 50 °C, 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C. Genotype analysis was performed on amplified samples using the ABI PRISM 7700 software, using the standard procedures for automated allelic discrimination.

Descriptive statistics were assessed for significant differences between cases and controls using the Student's *t*-test for comparison of means and χ^2 test for comparison of binomial proportions. MnSOD genotype data were assessed for deviations from Hardy–Weinberg proportions using the χ^2 goodness-of-fit test. Case–control comparisons using the combined control groups were used to assess main genotypic effects on ovarian cancer risk. Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained from univariate and multivariate unconditional logistic regression adjusting for age, parity and age at menarche. Case-only methods were used to estimate the genotype-by-nutrient interaction. Dietary variables were categorized as low or high based on the median value, and entered into polytomous regression models as independent variables with genotype modeled as the dependent variable with three categories. Survival time was calculated from date of diagnosis to date of death (from ovarian cancer) or censored at 1 September 2004 or death from another cause. The Kaplan–Meier technique was used to plot crude survival curves and estimate crude overall survival probabilities, and adjusted hazard ratios (HR) and 95% CIs were obtained from Cox regression models. Hazard ratios and 95% CIs were adjusted for age (10-year age groups), FIGO stage, histologic subtype, grade, and treatment with platinum-based chemotherapy. All statistical tests and *P*-values were two-tailed and statistical significance was assessed at the conventional level of less than 0.05. STATA 9.0 (Stata Corporation, College Station, TX) and Statistical Packages for Social Sciences for Windows, Version 10.0 (SPSS Inc., Chicago, IL) were used for statistical analyses.

Results

The distribution of Val9Ala genotypes in the combined group of controls was in Hardy–Weinberg equilibrium ($p>0.2$). Multivariate analysis showed no significant association between the Val9Ala polymorphism and ovarian cancer (Table 2). Further adjustment for oral contraceptive use in the subset of women with this information available did not appreciably alter the odds ratio, nor did restricting analyses to serous cases only (data not shown). Case-only analysis of genotype-by-nutrient interaction showed little evidence of a significant interaction between Val9Ala genotypes and consumption of dietary sources of anti-oxidants (Table 3). A marginally significant interaction between low vitamin C consumption and Val9Ala heterozygosity in both univariate and multivariate analyses was detected ($p=0.03$). However, given the lack of trend for an increasing number of alanine alleles, and the marginal *p*-value which would not withstand correction for multiple testing, it is likely that this result is due to chance.

The results of survival analyses are shown in Table 4. Carriers of the alanine variant had no survival advantage compared to valine homozygotes in either univariate or multivariate analysis.

Table 2
Case control analysis of ovarian cancer and MnSOD Val–9Ala polymorphism

Genotype	Controls, *N* (%)	Cases, *N* (%)	Univariate OR (95% CI), *p*-value	Adjusted[a] OR (95% CI), *p*-value	*P* trend[b]
Val–Val	276 (24.4)	123 (22.6)	1.00	1.00	
Val–Ala	546 (48.3)	273 (50.3)	1.12 (0.87–1.45), $p=0.4$	1.25 (0.90–1.73), $p=0.2$	
Ala–Ala	308 (27.3)	147 (27.1)	1.07 (0.80–1.43), $p=0.6$	1.17 (0.81–1.67), $p=0.4$	0.7
Ala carriers	854 (75.6)	420 (77.4)	1.10 (0.87–1.41), $p=0.4$	1.22 (0.89–1.66), $p=0.2$	

[a] Adjusted for age (at diagnosis for cases; at interview for controls), parity, and age at menarche.
[b] χ^2 test for trend in risk by increasing alanine allele.

Table 3
Case-only odds ratios for interaction between MnSOD genotype and dietary intake of anti-oxidants

Dietary intake [a]	Val–Val genotype (Ref.)	Val–Ala genotype	Ala–Ala genotype	Univariate OR (95% CI), *p*-value		Adjusted [b] OR (95% CI), *p*-value	
	N=68	*N*=160	*N*=84	Val–Ala vs. Val–Val	Ala–Ala vs. Val–Val	Val–Ala vs. Val–Val	Ala–Ala vs. Val–Val
Total fruit and vegetables							
High	32	88	35	1.00	1.00	1.00	1.00
Low	36	72	49	0.73 (0.41–1.28),	1.24 (0.65–2.37),	0.82 (0.45–1.50),	1.46 (0.74–2.88),
			p=0.4 [c]	*p*=0.3	*p*=0.5	*p*=0.5	*p*=0.3
Vitamin C							
High	28	91	38	1.00	1.00	1.00	1.00
Low	40	69	46	0.53 (0.30–0.94),	0.85 (0.44–1.62),	0.55 (0.30–1.00),	0.88 (0.45–1.74),
			p=0.1 [c]	*p*=0.03	*p*=0.6	*p*=0.05	*p*=0.7
Vitamin E							
High	29	91	36	1.00	1.00	1.00	1.00
Low	39	69	48	0.56 (0.32–1.00),	0.99 (0.52–1.89),	0.75 (0.41–1.39),	1.46 (0.73–2.90),
			p=0.8 [c]	*p*=0.05	*p*=1.0	*p*=0.4	*p*=0.3
β-carotene							
High	31	85	41	1.00	1.00	1.00	1.00
Low	37	75	43	0.74 (0.42–1.31),	0.88 (0.46–1.67),	0.79 (0.43–1.45),	0.91 (0.46–1.79),
			p=0.5 [c]	*p*=0.3	*p*=0.7	*p*=0.4	*p*=0.8

[a] Dietary variables are dichotomized as low and high based on median values among cases; total fruits and vegetables=7.5 servings/day, vitamin C=165.6 mg/day, vitamin E=9.3 mg/day, and β-carotene=5161.2 μg/day.
[b] Adjusted for age at diagnosis, oral contraceptive use, parity, age at menarche, family history of ovarian cancer.
[c] χ^2 test for adjusted linear trend.

The results of survival analyses stratified by high versus low vegetable and fruit intake (based on the median intake value) were also non-significant (high intake: adjusted $HR_{Val/Ala}$, 0.77; 95% CI, 0.38–1.57, $HR_{Ala/Ala}$, 0.78; 95% CI, 0.35–1.75; low intake adjusted $HR_{Val/Ala}$, 1.10; 95% CI, 0.55–2.20, $HR_{Ala/Ala}$, 0.90; 95% CI, 0.38–2.13).

Discussion

Our study is the first to investigate the role of the MnSOD Val9Ala polymorphism and its interaction with dietary anti-oxidants in ovarian cancer risk and survival. The results of Val9Ala association studies and cancer risk have been inconsistent, with the alanine allele associated with an increased risk of breast cancer [12] and prostrate cancer [13] in some studies, and a decreased risk of breast cancer [14] and lung cancer [15] in others. *In vitro* and animal studies [16], as well as computer-based predictive models [17] have demonstrated that the alanine-containing MnSOD precursor protein is more efficiently processed compared to the valine-containing protein, suggesting that the valine allele might increase cancer risk. However for purposes of comparison with previous studies, we analyzed the alanine allele as the risk allele. We found no altered risk and no survival benefit associated with the alanine allele.

The hypothesis that ovarian cancer is etiologically related to oxidative damage [4] and that dietary antioxidant intake may contribute to risk reduction has been extensively studied, with findings that both support [18] and refute [19] this hypothesis. If the Val9Ala genotype compromises MnSOD enzyme efficiency such that oxidative damage to the cell is increased, then it is plausible that such deleterious effects would be exacerbated in conjunction with low dietary antioxidant intake. We therefore investigated whether there was any interaction between dietary intake of antioxidants and MnSOD genotype but found no evidence of this. We have previously shown that diet may influence survival among ovarian cancer cases [20] and thus hypothesized that diet might modify the effect of MnSOD genotype on survival among women with ovarian cancer.

Table 4
Association between MnSOD Val–9Ala polymorphism and ovarian cancer survival

Genotype	Cases, *N* (%)	Deaths, *N* (%)	Crude 5-year survival (%)	Univariate (HR, 95% CI)	Adjusted HR [a] (95% CI)	*P* trend [b]
Val–Val	100 (22)	58	49	1.0	1.0	
Val–Ala	228 (51)	153	42	1.18 (0.87–1.60)	0.82 (0.59–1.14)	
Ala–Ala	119 (27)	73	46	1.03 (0.73–1.46)	0.91 (0.63–1.33)	0.9

[a] Adjusted for age, FIGO stage, histologic grade and subtype, platinum-based chemotherapy.
[b] χ^2 test for trend in risk by increasing alanine allele.

However we found no evidence that dietary intake modified survival according to MnSOD genotype. While our results appear convincingly null, we do acknowledge that our analyses were based on a limited sample of 312 cases with genotype and dietary data.

Our study did not replicate the previously reported twofold increase in risk of ovarian cancer associated with alanine homozygotes [6] although we had 99% power to detect an OR of this magnitude. We also had 80% power to detect ORs of 1.3 and 1.5 associated with MnSOD Val9Ala heterozygotes and alanine homozygotes, respectively. We cannot rule out the possibility that this polymorphism may be associated with even smaller risks of ovarian cancer, but suggest that these are best explored using large collaborative studies such as the Ovarian Cancer Association Consortium, which will provide much greater power to detect small effects, or those restricted to subgroups defined by tumor subtype or etiology.

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