Carcinogenesis vol.22 no.5 pp.827–829, 2001

SHORT COMMUNICATION

Association between manganese superoxide dismutase (*MnSOD*) gene polymorphism and breast cancer risk

Katja Mitrunen, Pia Sillanpää, Vesa Kataja[1], Matti Eskelinen[2], Veli-Matti Kosma[3], Simone Benhamou[4], Matti Uusitupa[5] and Ari Hirvonen[6]

Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Helsinki, [1]Department of Oncology, [2]Department of Surgery, [3]Department of Clinical Pathology and Forensic Medicine and [5]Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland and [4]Unit of Cancer Epidemiology (INSERM U521), Gustave-Roussy Institute, Villejuif, France

[6]To whom correspondence should be addressed at: Molecular Epidemiology Group, Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Topeliuksenkatu 41 a A, FIN-00250 Helsinki, Finland
Email: Ari.Hirvonen@occuphealth.fi

Superoxide dismutases play a key role in the detoxification of superoxide radicals and thus protect cells from damage induced by free radicals. Within mitochondria manganese superoxide dismutase (MnSOD) provides a major defence against oxidative damage by reactive oxygen species. Polymorphism in the mitochondrial targeting sequence of *MnSOD* has recently been associated with risk of breast cancer. We examined this in a study population consisting of 483 breast cancer cases and 482 controls, all of Finnish Caucasian origin. Odds ratios (OR) and 95% confidence intervals (95% CIs) were estimated by unconditional logistic regression. *MnSOD* genotypes containing the variant *A* allele were found to be associated with a 1.5-fold (95% CI 1.1–2.0) increased risk of breast cancer compared with those with the homozygous wild-type genotype (*MnSOD VV*). This finding supports the proposal that *MnSOD* genotypes may modify individual breast cancer risk.

Lifetime exposure to exo- and endogenous oestrogens appears to be closely related to development and progression of breast cancer. The role of cigarette smoking, alcohol and diet have also been extensively studied with controversial results (1). One potential mechanistic basis for these factors is through reactive oxygen species (ROS)-induced oxidative damage that has been related to the aetiology of human cancer (2).

Superoxide dismutases play a key role in the detoxification of superoxide radicals (O_2^-) thereby protecting cells from damage induced by free radicals (3). Within mitochondria manganese superoxide dismutase (MnSOD) provides a major defence against oxidative damage by ROS. It is synthesized in the cytosol and post-transcriptionally modified for transport into the mitochondrion (4). A variety of cancer cells are known to express reduced levels of antioxidant enzymes, especially MnSOD, when compared with their normal counterpart (5,6). Furthermore, the increased expression of *MnSOD* has been found to suppress the malignant phenotype of human breast cancer cells suggesting that *MnSOD* is a tumour suppressor gene in human breast cancer (7).

Mitochondrial targeting sequence (MTS) readily forms an amphiphilic helical structure, which is crucial for effective transport and processing of mitochondrial proteins. A one base pair transition (T→C) leads to a Val→Ala amino acid change at –9 position of MTS (8). This change, the prevalence of which has been found to differ among various ethnic groups (9), may result in a 'disease of distribution' where allocation rather than activity of an essential protein is faulty (10). In agreement with this, the *MnSOD AA* genotype has been associated with increased breast cancer risk, especially among pre-menopausal women with a low consumption of dietary sources of antioxidants (11).

This study, which is an extension of the Kuopio Breast Cancer Study (12), was undertaken to examine the role of the *MnSOD* genotype further in a Finnish Caucasian study population.

The cases consisted of women who had a suspicious breast lump or breast symptoms and lived in the catchment area of Kuopio University Hospital during the study period (from 1990 to 1995). They were referred by a physician for further examination, and were asked to participate in the study at the first hospital visit. Detailed data concerning socio-economic background, reproductive history, medical history, family history of breast cancer, current alcohol intake, smoking and body-size indicators were recorded during an interview with a study nurse, completed before any diagnostic procedures had begun (12). A total of 516 women were finally diagnosed with histologically confirmed breast cancer. Only 12 women later diagnosed with breast cancer refused to participate. The final participation rate for the cases was therefore 98%.

Lymphocyte DNA was available for 486 breast cancer patients. Three of the patients were excluded from the study because of poor quality of the sample DNA. Thus the final patient population included 483 incident breast cancer cases (44.3–91.6 years of age, mean 58.9 years).

Healthy population controls with no breast symptoms or previous breast problems and living in the study catchment area were drawn from the Finnish National Population Register. In all, 514 controls were interviewed in parallel with the cases, the final participation rate being 72%. Of these, lymphocyte DNA was available for 492 subjects. Four population controls having earlier breast cancer diagnosis, two controls of non-Finnish origin and four controls whose DNA was of poor quality were excluded from the study. Therefore, the final study population included 482 controls (37.5–77.2 years of age, mean 53.5 years).

Lymphocyte DNA (100 ng) extracted by standard techniques was used as a template in PCR-based restriction fragment length polymorphism (RFLP) assays performed essentially as described earlier (11). Briefly, the 107 bp PCR product was amplified using specific primers (5′-ACC AGC AGG CAG

Abbreviations: MnSOD, manganese superoxide dismutase; MTS, mitochondrial targeting sequence; OR, odds ratio; RFLP, restriction fragment length polymorphism; ROS, reactive oxygen species.

© Oxford University Press

CTG GCG CCG G-3′ and 5′-GCG TTG ATG TGA GGT TCC AG-3′), which create a restriction site for *Ngo*MIV (New England Biolabs) in the *MnSOD A* allele resulting in restriction fragments of 89 and 18 bp. The uncut 107 bp fragment identified the *MnSOD V* allele.

Positive and negative controls were used within each batch of PCR. The experimenter was unaware of each sample's case-control status. Two independent readers interpreted the RFLP results. All samples with ambiguous results, and a random selection of 10% of all samples, were repeated to ensure laboratory quality control. Because of unreadable genotype data for one control and three cases, results are presented for 482 controls and 479 cases.

Age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression. Women who reported natural menopause or had undergone bilateral oophorectomy were classified post-menopausal. Hysterectomized women with intact ovaries (ovary) (40 cases and 41 controls) and women for whom the details of the operation were unknown (six cases and two controls) were also classified post-menopausal if they were no longer menstruating and were older than 51 years (median for menopause in Finnish women). All the others were classified as pre-menopausal.

Interactions between *MnSOD* genotype and smoking, use of alcohol, use of oral contraceptives, post-menopausal use of oestrogen or use of supplement vitamins were assessed by the likelihood tests to compare the goodness of the fit of the models with and without the interaction term. Subjects who had in the past smoked daily for at least 3 months were considered as smokers. Ever smokers (ex- or current at the reference date) were further categorized to two levels of smoking exposure according to the approximate median value for smoking years (15 years) and daily consumption (10 cigarettes) in the control population. Daily tobacco consumption was similar in cases and controls (11.2 cigarettes/day versus 9.5 cigarettes/day, $P = 0.099$) as was the duration of smoking (16.1 years versus 14.5 years, $P = 0.250$).

Women who reported taking vitamin supplements (A, C or E vitamins) at least three times per week for longer than 6 months were considered as users of vitamin supplements. Average daily consumption of tobacco, use of oral contraceptives and use of post-menopausal oestrogen were considered as categorical or continuous variables.

Individuals carrying the homozygous *MnSOD VV* genotype served as a reference category for all separate analysis of the genotype. All reported *P* values were two-sided, and a result was considered significant when $P < 0.05$.

In a previous study, we found waist to hip ratio, first degree family history of breast cancer and history of benign breast disease were positively associated with breast cancer risk, whereas parity and use of oral contraceptives showed an inverse association compared with women without these risk factors (13).

The genotype distributions in the control population were in agreement with those predicted under the conditions of Hardy–Weinberg equilibrium ($P = 0.652$). A 1.5-fold (95% CI 1.1–2.0) risk of breast cancer was found for individuals carrying the *MnSOD A* allele-containing genotypes compared with the *MnSOD VV* genotype (Table I). A similar risk approaching statistical significance was also observed in the sole previous study on breast cancer risk and *MnSOD* polymorphism (OR 1.8, 95% CI 0.9–3.6) (11). The most remarkable risk in that study, however, was associated with the *MnSOD AA* genotype compared with the *VV* genotype (OR 4.3, 95% CI 1.7–10.8) in pre-menopausal women. In our study, this comparison revealed no statistically significant association (OR 1.4, 95% CI 0.7–2.5), possibly because of lack of statistical power.

Table I. Association between *MnSOD* genotype and development of breast cancer according to menopausal status at diagnosis of the case patient

Genotype	Cases *n* (%)	Controls *n* (%)	OR[a] (95% CI)
All			
MnSOD VV	124 (25.9)	153 (31.7)	1.0 (ref.)
MnSOD VA	255 (53.2)	231 (47.9)	1.5 (1.1–2.0)
MnSOD AA	100 (20.9)	98 (20.3)	1.4 (0.9–2.0)
MnSOD VA/AA	355 (74.1)	329 (68.3)	1.5 (1.1–2.0)
Pre-menopausal			
MnSOD VV	46 (28.0)	62 (30.4)	1.0 (ref.)
MnSOD VA	82 (50.0)	106 (52.0)	1.1 (0.7–1.8)
MnSOD AA	36 (22.0)	36 (17.6)	1.4 (0.7–2.5)
MnSOD VA/AA	118 (72.0)	142 (69.6)	1.2 (0.7–1.8)
Post-menopausal			
MnSOD VV	78 (24.8)	91 (32.7)	1.0 (ref.)
MnSOD VA	173 (54.9)	125 (45.0)	1.9 (1.2–2.8)
MnSOD AA	64 (20.3)	62 (22.3)	1.4 (0.9–2.3)
MnSOD VA/AA	237 (75.2)	187 (67.3)	1.7 (1.2–2.5)

[a]ORs and 95% CIs adjusted for age.

Table II. Distribution of *MnSOD* genotypes (case/control) and ORs[a] (95% CI) for breast cancer according to presumed risk and protective factors among postmenopausal women

Risk/ protective factors	Genotype			
	MnSOD VV	*MnSOD VA*	*MnSOD AA*	*MnSOD VA/AA*
Alcohol consumption				
No	1.0 (ref.)	1.6 (0.9–2.7)	1.0 (0.6–2.0)	1.4 (0.8–2.3)
	56/43	118/72	43/37	161/109
Yes	1.0 (ref.)	2.3 (1.2–4.4)	2.0 (0.9–4.5)	2.2 (1.2–4.1)
	20/48	54/53	21/25	75/78
History of smoking				
No	1.0 (ref.)	1.6 (1.0–2.5)	1.2 (0.7–2.1)	1.5 (1.0–2.3)
	64/70	141/104	53/53	194/157
Yes	1.0 (ref.)	4.0 (1.4–11.2)	3.2 (0.9–11.0)	3.7 (1.4–9.9)
	12/21	30/21	11/9	41/30
Use of oral contraceptives				
No	1.0 (ref.)	1.6 (1.0–2.5)	1.2 (0.7–2.2)	1.5 (0.9–2.3)
	64/57	138/91	51/43	189/134
Yes	1.0 (ref.)	3.4 (1.4–8.0)	2.4 (0.9–6.5)	3.0 (1.3–6.8)
	10/34	33/34	13/19	46/53
Postmenopausal estrogen use[b]				
No	1.0 (ref.)	1.7 (1.0–2.9)	0.9 (0.5–1.7)	1.4 (0.9–2.3)
	57/50	121/71	40/43	161/114
Yes	1.0 (ref.)	2.2 (1.1–4.5)	3.3 (1.4–7.6)[c]	2.5 (1.3–4.8)
	17/41	49/54	24/19	73/73
Antioxidant supplements				
No	1.0 (ref.)	1.8 (1.2–2.9)	1.2 (0.7–2.1)	1.6 (1.0–2.5)
	60/74	141/103	52/57	193/160
Yes	1.0 (ref.)	2.4 (0.8–6.9)	3.3 (0.7–15.0)	2.5 (0.9–7.1)
	15/17	31/22	12/5	43/27

[a]ORs and 95% CIs adjusted for age.
[b]*P* for interaction 0.02.
[c]*P* for trend 0.01.

No remarkable difference was seen when the association between the *MnSOD* genotype and breast cancer risk was considered by the stage of the disease or by the oestrogen receptor status (data not shown).

When the risk associated with the *MnSOD A* allele was studied stratified by factors suspected to have a role in the production of ROS or in their detoxification, no significant associations were seen when pre- and post-menopausal women were considered together. However, after stratification by menopause, significant interaction was found between the *MnSOD* genotypes and post-menopausal use of oestrogen; women who had used oestrogen replacement therapy and carrying a *MnSOD A* allele containing genotypes had a 2.5-fold higher risk of breast cancer (95% CI, 1.3–4.8; *P* for interaction 0.02) (Table II).

No other significant interactions were seen between genotype and risk factors studied. Nevertheless, an increased risk was observed among post-menopausal women who reported having smoked in the past and carrying a *MnSOD A* allele-containing genotype (OR 3.7, 95% CI 1.4–9.9). The highest risk was seen for women who carried the *MnSOD A* allele containing genotypes and who had been smoking for longer than 15 years or >10 cigarettes/day with respective unadjusted ORs of 5.1 (95% CI 1.4–18.4) and 5.5 (95% CI 1.3–23.4) (data not shown). Adjustment yielded higher but unstable estimates, with a wide CI, due to the small number of 'heavy smokers' in the sample. In contrast, no difference was seen among ex- or current smokers (data not shown).

Similarly, in post-menopausal women the effect of *MnSOD A* allele-containing genotypes was more pronounced among current users of alcohol and women who had in the past used oral contraceptives (Table II). These associations were not modified by the duration of use.

The strengths of this study are its relatively large size, genetic homogeneity of the study population, and good background information on both reproductive history and many life style factors, whereas one of its limitations might be the absence of information on dietary habits of the study subjects, restricting us from assessing the risk modification by diet, as was done by Ambrosone *et al.* (11). However, we could examine the effect of intake of vitamin supplements as was also done in the above study (11), where pre-menopausal women not taking vitamin C or α-tocopherol were shown to be at increased risks. We could not, however, see any effect when the use of A, C or E vitamins were considered together. This might be explained, at least in part, by the low numbers of subjects using these vitamins.

It should also be noted that all analyses stratified by menopausal status were based on substantially lower numbers of subjects in each strata, lowering the power of the analyses. This might at least partly explain why most statistically significant associations were seen in subjects with heterozygous *MnSOD VA* genotype, while no trend of increasing risk was seen among subjects carrying the *MnSOD AA* genotype. It may also be one explanation for the lack of any significant associations among the pre-menopausal women. Further studies with larger sample sizes to offer sufficient statistical power also for the subgroup analyses are therefore evidently needed before any strict conclusions can be drawn on this issue.

According to this hypothesis, the polymorphism in MTS of MnSOD affects the mitochondrial targeting of the enzyme, modifying the ability of mitochondria to defend against oxidative stress by ROS. However, the amphiphilic helical structure essential for correct transport is predicted for the *MnSOD A* allele, which was the risk allele both in this and in the previous study by Ambrosone *et al.* (11). The possible explanation for the negative role of the *MnSOD A* allele may be the simultaneous accumulation of H_2O_2, at least in the absence of H_2O_2 scavenging enzymes such as glutathione peroxidase and catalase. In the presence of transition metals, this in turn gives rise to generation of potent free radicals and hence mitochondrial damage. Furthermore, as discussed by Ambrosone *et al.* (11), the prediction of an altered cellular location of the *MnSOD* is based on a limited statistical model and should thus be interpreted with caution.

To conclude, although no strict conclusions can be drawn before real data on the biological consequences of the *MnSOD* variants is available, our results support the hypothesis that *MnSOD* genotypes may modify individual breast cancer risk. This remains to be corroborated in future studies.

Acknowledgements

We thank our colleagues at the Kuopio Cancer Research Center, Ms A.K.Lyytinen, R.N., for data collection. The work was supported by the Academy of Finland, the Finnish Konkordia Foundation, and EVO funds from Kuopio University Hospital.

References

1. Kelsey,J.L. and Gammon,M.D. (1991) The epidemiology of breast cancer. *CA Cancer J. Clin.*, **41**, 146–165.
2. Emerit,I. (1994) Reactive oxygen species, chromosome mutation, and cancer: possible role of clastogenic factors in carcinogenesis. *Free Radic. Biol. Med.*, **16**, 99–109.
3. Fridovich,I. (1995) Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.*, **64**, 97–112.
4. Wispe,J.R., Clark,J.C., Burhans,M.S., Kropp,K.E., Korfhagen,T.R. and Whitsett,J.A. (1989) Synthesis and processing of the precursor for human mangano-superoxide dismutase. *Biochim. Biophys. Acta*, **994**, 30–36.
5. Oberley,L.W. and Buettner,G.R. (1979) Role of superoxide dismutase in cancer: a review. *Cancer Res.*, **39**, 1141–1149.
6. Oberley,L.W. and Oberley,T.D. (1988) Role of antioxidant enzymes in cell immortalization and transformation. *Mol. Cell Biochem.*, **84**, 147–153.
7. Li,J.J., Oberley,L.W., StClair,D.K., Ridnour,L.A. and Oberley,T.D. (1995) Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase. *Oncogene*, **10**, 1989–2000.
8. Shimoda-Matsubayashi,S., Matsumine,H., Kobayashi,T., Nakagawa-Hattori,Y., Shimizu,Y. and Mizuno,Y. (1996) Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem. Biophys. Res. Commun.*, **226**, 561–565.
9. Van Landeghem,G.F., Tabatabaie,P., Kucinskas,V., Saha N. and Beckman,G. (1999) Ethnic variation in the mitochondrial targeting sequence polymorphism of MnSOD. *Hum. Hered.*, **49**, 190–193.
10. Rosenblum,J.S., Gilula,N.B. and Lerner,R.A. (1996) On signal sequence polymorphisms and diseases of distribution. *Proc. Natl Acad. Sci. USA*, **93**, 4471–4473.
11. Ambrosone,C.B., Freudenheim,J.L., Thompson,P.A., Bowman,E., Vena,J.E., Marshall,J.R., Graham,S., Laughlin,R., Nemoto,T. and Shields,P.G. (1999) Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Res.*, **59**, 602–606.
12. Mannisto,S., Pietinen,P., Pyy,M., Palmgren,J., Eskelinen,M. and Uusitupa,M. (1996) Body-size indicators and risk of breast cancer according to menopause and estrogen-receptor status. *Int. J. Cancer*, **68**, 8–13.
13. Mitrunen,K., Jourenkova,N., Kataja,V., Eskelinen,M., Kosma,V.M., Benhamou,S., Vainio,H., Uusitupa,M. and Hirvonen,A. (2000) Steroid metabolism gene *CYP17* polymorphism and the development of breast cancer. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 1343–1348.

Received November 10, 2000; revised and accepted January 29, 2001