# Germline Variation in Superoxide Dismutase-2 (SOD2) and Survival Outcomes after Radiation Therapy for Prostate Cancer: Results from a Test and Validation Set Analysis

HHS Public Access

Author manuscript

*Clin Genitourin Cancer*. Author manuscript; available in PMC 2016 September 27.

Published in final edited form as:

*Clin Genitourin Cancer*. 2015 August ; 13(4): 370–377.e1. doi:10.1016/j.clgc.2014.12.018.

# Germline Variation in Superoxide Dismutase-2 (*SOD2*) and Survival Outcomes after Radiation Therapy for Prostate Cancer: Results from a Test and Validation Set Analysis

**Danielle N. Margalit**[1], **Kristina M. Jordahl**[2], **Lillian Werner**[3], **Xiaodong Wang**[4], **Mary Gwo-Shu Lee**[4], **Kathryn L. Penney**[2,5], **Julie L. Batista**[2,5], **Neil E. Martin**[1], **June M. Chan**[6], **Philip W. Kantoff**[4], **Meir J. Stampfer**[2,5], **Paul L. Nguyen**[1,*], and **Lorelei A. Mucci**[2,5,*]

[1]Department of Radiation Oncology, Brigham & Women's Hospital/Dana-Farber Cancer Institute, Boston, MA

[2]Department of Epidemiology, Harvard School of Public Health, Boston, MA

[3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA

[4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

[5]Channing Division of Network Medicine, Department of Medicine, Brigham & Women's Hospital and Harvard Medical School, Boston, MA

[6]Department of Epidemiology & Biostatistics, University of California San Francisco, San Francisco, CA

## Abstract

**Background—**Genetic variants in antioxidant pathways may decrease the efficacy of radiation therapy (RT) by suppressing the generation of reactive oxygen species (ROS). We studied the association between single nucleotide polymorphisms (SNPs) in the antioxidant gene superoxide dismutase-2 (*SOD2*) and cancer-specific outcomes after RT.

**Methods—**Among 816 prostate cancer patients who received radiation as primary therapy from the Physicians' Health Study and the Health Professionals Follow-up Study, we evaluated the association of 7 tagging SNPs in *SOD2* with lethal prostate cancer (death from prostate cancer or distant metastasis among living patients). We sought to validate findings in a separate cohort of 612 prostate cancer patients treated with RT with a higher proportion of intermediate and high-risk



Corresponding author: Danielle N. Margalit, MD MPH, Dana-2, 450 Brookline Avenue, Boston, MA, 02215, USA. Phone: 1-617-632-3591. Fax: 1-617-632-4247, dmargalit@lroc.harvard.edu.

*These authors share last authorship

**Conflict of interest statement:** The authors have no actual or potential conflicts of interest or financial disclosures to report.

Gleason scores at the Dana-Farber Cancer Institute. Genetic effects were analyzed using a co-dominant model, using the genotype homozygous for the major allele as baseline.

**Results—**Among patients who underwent RT in the test cohort, there was a significant association between three of the seven *SOD2* SNPs and lethal prostate cancer: rs6917589 (overall p-value =0.006), rs2758331 (p=0.04) and the functional valine to alanine polymorphism in rs4880 (p=0.04). These SNPs were not associated with outcome among men who had undergone prostatectomy. The associations were not replicated in the validation cohort.

**Conclusion—**Germline genetic variation in the *SOD2* gene may be a predictive biomarker of response to radiation therapy for prostate cancer but is not consistently associated with outcome after radiation therapy across prostate cancer cohorts with different clinical characteristics.

### Keywords

antioxidant; radiation therapy; superoxide dismutase; SOD2; free radicals; reactive oxygen species; prostate cancer outcomes

## INTRODUCTION

Germline variation in antioxidant pathways may alter the effect of cancer therapies that rely on the generation of cytotoxic reactive oxygen species (ROS). Somatic alterations in the antioxidant environment are also postulated to result in enhanced cancer cell survival (1). There is growing interest in molecular-based strategies that target antioxidant pathways to promote cancer cell killing via oxidative stress (2–4). Radiation therapy generates ROS that mediate DNA damage and other downstream effects on cancer cells (5). Patient germline variability in endogenous antioxidant enzymes involved in neutralizing ROS may explain variability in cancer-specific outcomes after radiation therapy (RT). For example, patients with increased capacity for neutralizing ROS may receive less benefit from RT compared to patients with impaired ability to neutralize cytotoxic ROS.

Superoxide dismutase-2 (SOD2) is a mitochondrial antioxidant enzyme that is an important ROS scavenger. SOD2 reduces superoxide anion to hydrogen peroxide and oxygen, which is then converted to water by catalase (CAT) and glutathione peroxidase (GPX) (Figure 1). Overexpression of mitochondrial SOD was previously shown to protect cells from radiation-induced neoplastic transformation (6) and decreased levels of SOD increased the radiosensitivity of prostate cancer cells *in vitro* (7). A specific polymorphism in codon 16 of *SOD2*, rs4880, results in a valine to alanine amino acid change and is postulated to decrease mitochondrial ROS by causing more efficient transport of the enzyme into the mitochondria (8, 9). The polymorphism would be expected to decrease the effectiveness of cancer therapies such as radiation therapy, which rely on formation of ROS. Polymorphisms in *SOD2* were previously shown to be associated with late toxicity after radiation therapy for prostate cancer (10), breast cancer (11) and head and neck cancer (12).

There is conflicting data on the prognostic significance of *SOD2* polymorphisms and survival after cancer therapy (13, 14). This study sought to validate the association between *SOD2* polymorphisms and cancer outcomes after radiation therapy for prostate cancer. We hypothesized that germline genetic variation in *SOD2* is associated with outcome after RT

and that the functional rs4880 polymorphism is associated with adverse prostate cancer outcomes.

## MATERIALS AND METHODS

### Patients and Outcomes

The test cohort was comprised of 816 participants from two prospective cohort studies, the Physicians' Health Study (PHS; 1982 – 2009 N=387) and the Health Professionals Follow-up Study (HPFS; 1993–2010 N=429). The PHS (15–17) was a 2x2 randomized double-blind, placebo-controlled trial that began in 1982 and enrolled 22,071 U.S. male physicians ages 40–84 years to take 325 mg aspirin and/or 50 mg beta-carotene every other day or placebo. Participants were free from diagnosed cancer at enrollment and were followed with yearly questionnaires and postcards at 6 month intervals to ascertain endpoints, including prostate cancer. At baseline, 14,916 (68%) participants provided blood before randomization and cancer diagnosis. The prospective HPFS enrolled 51,529 male medical professionals in 1986 to investigate the causes of cancer and heart disease. These cohort participants are subsequently followed with biennial questionnaires designed to collect information about medical diagnoses and lifestyle factors. Response rates to the follow-up surveys are high at approximately 96% and 18,018 participants provided a blood sample between 1993 and 1995.

When a participant reported a diagnosis of prostate cancer, hospital records and pathology reports were requested and study physicians verified diagnosis by reviewing medical records and pathology reports to determine the Gleason grade, stage, and prostate specific antigen (PSA) level at diagnosis. This study includes men in the PHS and HPFS blood cohorts who were diagnosed with prostate cancer between 1982 and 2010 and who underwent radiation therapy. Participants were excluded if radiation therapy was not their primary treatment or if their first treatment was radical prostatectomy.

For comparison, we also analyzed the association of polymorphisms in *SOD2* in patients who underwent radical prostatectomy without radiation therapy, reasoning that the genetic variations would have no impact after surgical intervention. This separate cohort included 1094 patients from the PHS (N=555) and the HPFS (N=539). These studies were approved by the institutional review board at the Harvard School of Public Health and Partners Health Care.

The validation cohort consisted of patients from the Prostate Clinical Research Information System (CRIS; 1990–2008 N=612) at the Dana-Farber Cancer Institute. CRIS consists of a central secure data repository of patient data, including baseline clinical and disease characteristics and information about treatment and outcomes. All prostate cancer patients at Dana Farber Cancer Institute and Brigham and Women's Hospital were offered enrollment and 647 patients were initially identified for the validation cohort. Selected patients had prostate cancer, were treated with external beam radiation or brachytherapy, consented to provide information and tissue, and donated blood for research purposes. Patients were excluded if they had lymph node or distant metastases prior to radiation therapy, or if the samples failed > 50% of the genotyping assays.

For the test cohort, the primary outcome was time to development of lethal prostate cancer, defined as the time from initiation of radiation therapy (RT) to prostate-cancer-specific death or distant metastasis among living participants. Outcomes, including cause of death, were verified via death certificates and medical record review. Since it was not routinely verified in the PHS, we did not use biochemical recurrence as an outcome in the test cohort. For the validation cohort, the primary analysis evaluated the association between SNP genotypes and time to distant metastasis, which was defined as the time from the initiation of RT to the time when metastases developed. Due to shorter follow-up in the validation cohort, prostate-cancer death was not used as the primary outcome. As a secondary analysis, we also evaluated the association with time to biochemical recurrence. Time to biochemical recurrence was defined as the time from the start of radiation therapy to the time when nadir +2ng/mL occurred or to time of salvage therapy. If the outcome of interest did not occur, follow-up was censored on the last PSA date.

## Genotyping

We characterized one candidate SNP (rs4880) and 6 tagging SNPs from *SOD2* that were selected to capture genetic variation across the *SOD2* gene, including 5kb upstream and downstream, with an average $r^2 > 0.80$ (Tagger, http://www.broadinstitute.org/mpg/tagger/, using HapMap Release 21, CEU analysis panel: Utah residents of Northern and Western European Ancestry). For the test cohort, genotyping was performed at the Harvard Medical School – Partners Healthcare Center for Genetics and Genomics after extraction of DNA from whole blood using Biotrove Open Genetics and Genomics with a standard QIAmp kit (QIAGEN Inc. Chatsworth, CA) protocol. All SNPs had greater than 90% completion and the concordance was greater than 99% for blinded quality control samples. All SNPs were in Hardy-Weinberg equilibrium.

For the validation cohort, all DNA samples were extracted from patients' peripheral whole blood by QIAamp DNA Blood mini kit (QIAGEN Inc.) according to the manufacturer's instruction. Genotyping was performed at the core facility of Boston Children's Hospital using Sequenom iPLEX matrix–assisted laser desorption/ionization time-of-flight mass spectrometry technology. Approximately 5% of randomly selected duplicates were included as the quality control. All SNPs had greater than 99% genotype passing rates and no discrepancy between duplicates was observed in the genotyping data. Laboratory personnel were blinded to all case status information.

## Statistical Methods

Patient clinical and disease characteristics at the time of diagnosis were summarized by median and inter-quartile range for continuous variables and by number and percentage for categorical variables. For both the test and validation cohorts, we analyzed the genetic effects of *SOD2* SNPs using the co-dominant model, where the heterozygous and homozygous minor allele genotypes were treated as separate categories and compared to the homozygous major allele genotype. For minor alleles with less than 10% frequency in the cohorts, we combined the minor homozygous with the heterozygous genotypes. The co-dominant model was used as it makes fewer assumptions about the nature of the effect of the minor allele on outcome as compared to the additive model.

Cox proportional hazards models were used to assess the unadjusted and adjusted association between SNP and outcome and were used to calculate hazard ratios and associated 95% confidence intervals. The adjusted models included biopsy Gleason score, log-transformed PSA at diagnosis, clinical stage, and age at treatment. The median age of diagnosis and treatment were the same. For the test cohort, year of diagnosis and cohort (PHS or HPFS) were also used as adjustment covariates, and missing values for the clinical variables used in the adjusted models were imputed using Multiple Imputation for Chained Equations (MICE) in *R*. The use of hormonal therapy was included in the adjusted model for only the validation cohort.

All reported p-values are 2-sided, with Bonferroni-corrected $p<0.007$ considered statistically significant and $p<0.05$ considered nominally significant. SAS version 9.3 (SAS institute Inc, Cary, North Carolina) and *R* version 3.0.2 were used for all analyses.

## RESULTS

Table 1 shows the patient characteristics from the test (N=816) and validation cohorts (N=612). Patients in the test cohort were older (median age 73 versus 64 years in the validation cohort) and had longer follow-up compared to the validation cohort (median 10.2 years versus 6.8 years). They were more likely to have low grade Gleason ≤6 tumors (60%) and to be treated in an earlier time period than the validation cohort where most patients had higher risk Gleason ≥7 (43%) or Gleason 8–10 (28%) tumors. As shown in Table 2, the minor allele frequencies for the 7 polymorphisms in *SOD2* were similar among the two cohorts. Three of the SNP's (rs4880, rs2758331, rs2758329) were in linkage disequilibrium with $r^2 \geq 0.8$.

During follow-up in the PHS and HPFS cohorts, there were 77 lethal prostate cancer events, of which 52 were cancer deaths and 25 were distant metastases among living patients. Known prognostic factors, including biopsy Gleason score (p-value < 0.001), log PSA (p-value = 0.008), clinical TNM-stage (p-value < 0.001), and year of diagnosis (p-value < 0.001) were associated with lethal prostate cancer. Table 3 shows that three of the seven SNPs were statistically significantly associated with the composite enpoint of prostate-cancer death or metastases among living participants, at $p<0.05$. rs6917589 polymorphism was associated with risk of lethal prostate cancer (p=0.006). Carriage of the C allele in rs4880, which results in the valine to alanine isoform of the enzyme, was associated with a nominally statistically significant decrease in risk of lethal prostate cancer (HR 0.37 for homozygous C/C and HR 0.84 for T/C genotype, p-value = 0.04) as compared to the T/T genotype. This borderline association was also observed for the minor allele genotypes amongst the other two tagging SNPs in linkage disequilibrium with rs4880 (rs2758331 and rs2758329).

In the cohort of patients who underwent radical prostatectomy for prostate cancer (N=1094), the median age at prostatectomy was 65 years and the median PSA at diagnosis was 6.2 ng/mL [IQR 4.7, 9.7]. In this cohort, 65% of patients had Gleason 6 or less and 94% of patients had clinical T1/2 tumors. With a median follow-up of 12 years, there were 71 occurrences of lethal prostate cancer, of which 43 were from prostate cancer deaths and 28

were from distant metastases among living patients. There was no association between any of the seven SNPs in *SOD2* and lethal prostate cancer outcome after adjustment for age at radical prostatectomy, clinical TNM stage, log PSA, biopsy Gleason score, year of diagnosis, and cohort (Table 3).

We further examined the association of the 7 SNPs in *SOD2* with prostate cancer recurrence and with development of metastatic disease in a separate higher risk cohort of prostate cancer patients undergoing radiation from the Dana-Farber Cancer Institute (N=612). The median follow-up time was 6.8 years (range 2 months – 20 years) from the initiation of radiation therapy. There were 277 patients that experienced biochemical recurrence, with a median time to biochemical recurrence of 4.5 years (95% CI: 3.9–5.2 years). Distant metastasis was also assessed as an outcome of interest based on a total of 168 patients that developed distant metastases and had a median time to distant metastasis of 11 years (95% CI: 10.4–13.5 years). In adjusted and unadjusted analyses, there was no association between rs6917589, rs4880, or other SNPs in *SOD2* and distant metastasis or biochemical recurrence (Table 4 and Supplementary Table 1).

## DISCUSSION

In a cohort of patients with predominantly lower risk prostate cancer that was treated with definitive radiation therapy, the *SOD2* rs6917589 was associated with risk of lethal prostate cancer. There were borderline statistically significant associations between rs2758331 and the functional *SOD2* rs4880 polymorphism and lethal prostate cancer in the test cohort. Of note, these three *SOD2* polymorphisms were not predictive of cancer-specific outcomes after radical prostatectomy.

The initial finding was not reproduced in a cohort of men with a higher proportion of intermediate to high-grade Gleason scores, where there was no association between any *SOD2* polymorphism and risk of biochemical recurrence or distant metastasis. This study comes after attention has focused on the lack of reproducibility of candidate gene association studies (18, 19). The RAPPER study included 637 patients that received radical prostate radiotherapy and it rigorously assessed the association between toxicity outcomes and 92 SNPs in 46 genes which had been previously reported to be statistically significantly associated with radiation toxicity. The study failed to reproduce any of the findings, but did report borderline statistical significance for the *SOD2* rs4880 (20). This current study benefits from having a total of 1,428 patients treated with radiation therapy and is the largest study to date to investigate the relationship between candidate gene polymorphisms and prostate cancer outcome after radiation therapy.

While it is possible that our initial observations of statistically significant associations for *SOD2* SNPs and outcomes were due to chance, it is also possible that differences in the study population, follow-up times, available outcomes, and clinical variables may also account for the lack of consistent results in the validation cohort. For example, patients in the validation cohort tended to be younger and to have more intermediate-risk disease than the older, predominantly low-risk patients in the test cohort. Also, the test cohort had substantially longer follow-up than the validation cohort. Androgen deprivation therapy

(ADT) was also commonly used in the validation cohort and is estimated to have been used much less often in the test cohort. The endpoints were also different. The test cohort utilized lethal prostate cancer as the outcome, with the majority of events being death from prostate cancer. Distant failure was not used as a separate endpoint in the first cohort due to the low number of verified self-reported events which was potentially due to less use and availability of post-treatment PSA monitoring or radiographic imaging to detect distant metastases compared with the more modern validation cohort. In the validation cohort, biochemical recurrence and distant metastasis were validated via medical records and were deemed the most appropriate as few deaths from cancer had occurred by end of follow-up. Data were not available for local recurrence after radiation therapy due to a lack of consistent screening and reporting of local recurrence in the test cohort. Lastly, many of the prostate biopsies from the test cohort were assigned a Gleason score during an earlier time period than the DFCI cohort. We previously reported that there is an upgrading in Gleason score after modern standardized review of the original biopsy specimens from these cohorts (21), making it challenging to compare the distribution of Gleason scores across the test and validation cohorts.

The *SOD2* rs4880 T/C polymorphism has been well studied and postulated to result in increased ability to neutralize ROS due to more efficient uptake into the mitochondrial matrix (22). It has been associated with aggressive prostate cancer incidence among men with low antioxidant nutritional intake (23, 24). However, there are conflicting data regarding the association between rs4880 and toxicity after radiation therapy. Some studies identified an association of rs4880 with increased risk of subcutaneous fibrosis in breast cancer patients that underwent radiation therapy (11) and with grade ≥3 side effects in predominantly breast cancer and head and neck cancer populations (13). Another study by Green *et al.* refuted the association between *SOD2* and radiotherapy complications in breast cancer patients (14). Our study did not find a reproducible association between 7 of the *SOD2* SNPs and prostate cancer outcomes, but there was a suggestion of increased survival after radiation therapy for the rs4880 polymorphism and of decreased survival after radiation therapy for the rs6917589 polymorphism.

Since the interaction between *SOD2* and the tumor microenvironment is more complex than a single enzymatic reaction, pathway analysis of SNPs may yet detect clinically significant associations by taking into account other key enzymes involved in regulating oxidative stress. For example, as shown in Figure 1, after *SOD2* catalyzes the conversion of superoxide anion to hydrogen peroxide, the myeloperoxidase enzyme catalyzes the conversion of hydrogen peroxide to hydrochlorous acid, which is another oxidizing agent that may cause a net effect of increased ROS. Alternatively, catalase and glutathione peroxidase may catalyze conversion of hydrogen peroxide to neutral species. Therefore, the overall effect of *SOD2* polymorphisms may be dependent on the activity of *MPO*, *CAT*, *GPX* and other factors that alter the local ROS concentration. The model may also need to take the nutritional status of the patient into account, as our collaborative group has previously reported an interaction between antioxidant status, such as plasma selenium, and a SOD polymorphism as related to the incidence of aggressive prostate cancer (23, 25).

This study benefits from a large sample size and two diverse cohorts to independently assess the association between *SOD2* polymorphisms and prostate cancer outcome after radiation therapy. Moreover, in the test cohort, we were able to make comparisons with men who were undergoing radical prostatectomy. A limitation of the study is that we examined only germline polymorphisms and therefore cannot assess the genetic changes within the tumor that may affect tolerance to oxidative stress. We also were not able to directly measure the degree of ROS within the tumor or stroma. Lastly, a pathway analysis may improve the ability to determine the complex interaction between *SOD2* polymorphisms and other genes involved in regulating antioxidant stress.

## CONCLUSION

In summary, the study shows that most common germline polymorphisms in the *SOD2* enzyme are unlikely to have a clinically significant impact on all patient outcome after radiation therapy when treated individually. Though not validated, genetic variants in *SOD2* may have an effect that is specific to low-risk prostate cancer patients, and merits further study.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

**Grant support:** This work was supported by the National Institutes of Health (T32 CA09001 to DNM and JLB, Principal Investigator MJS; CA-34944, CA-40360, CA-097193, CA055075, CA131945, CA136578, CA141298, CA176726 , HL-26490, and HL-34595 for the Physicians' Health Study), the Department of Defense Prostate Cancer Research Program (W81XWH-12-1-0072) to JLB, CA-106947 for the HPFS, the Dana-Farber Cancer Institute Mazzone Awards Program to JLB, and the Prostate Cancer Foundation (LAM, KLP, NEM).

The authors thank the participants and staff of the PHS and HPFS for their valuable contributions. In addition, they also thank the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. We thank the patients who enrolled in the DFCI Cohort.

## References

1. Schafer ZT, Grassian AR, Song L, Jiang Z, Gerhart-Hines Z, Irie HY, et al. Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. Nature. 2009; 461:109–13. [PubMed: 19693011]
2. Badr CE, Van Hoppe S, Dumbuya H, Tjon-Kon-Fat LA, Tannous BA. Targeting cancer cells with the natural compound obtusaquinone. Journal of the National Cancer Institute. 2013; 105:643–53. [PubMed: 23479453]
3. Scarbrough PM, Mapuskar KA, Mattson DM, Gius D, Watson WH, Spitz DR. Simultaneous inhibition of glutathione- and thioredoxin-dependent metabolism is necessary to potentiate 17AAG-induced cancer cell killing via oxidative stress. Free radical biology & medicine. 2012; 52:436–43. [PubMed: 22100505]
4. Andringa KK, Coleman MC, Aykin-Burns N, Hitchler MJ, Walsh SA, Domann FE, et al. Inhibition of glutamate cysteine ligase activity sensitizes human breast cancer cells to the toxicity of 2-deoxy-D-glucose. Cancer research. 2006; 66:1605–10. [PubMed: 16452219]
5. Hall, EJ.; Giaccia, AJ. Radiobiology for the radiologist. 6. Philadelphia: Lippincott Williams & Wilkins; 2006.

6. St Clair DK, Wan XS, Oberley TD, Muse KE, St Clair WH. Suppression of radiation-induced neoplastic transformation by overexpression of mitochondrial superoxide dismutase. Molecular carcinogenesis. 1992; 6:238–42. [PubMed: 1485915]
7. Josson S, Xu Y, Fang F, Dhar SK, St Clair DK, St Clair WH. RelB regulates manganese superoxide dismutase gene and resistance to ionizing radiation of prostate cancer cells. Oncogene. 2006; 25:1554–9. [PubMed: 16261162]
8. Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. 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. Biochemical and biophysical research communications. 1996; 226:561–5. [PubMed: 8806673]
9. Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics. 2003; 13:145–57. [PubMed: 12618592]
10. Burri RJ, Stock RG, Cesaretti JA, Atencio DP, Peters S, Peters CA, et al. Association of single nucleotide polymorphisms in SOD2, XRCC1 and XRCC3 with susceptibility for the development of adverse effects resulting from radiotherapy for prostate cancer. Radiation research. 2008; 170:49–59. [PubMed: 18582155]
11. Andreassen CN, Alsner J, Overgaard M, Overgaard J. Prediction of normal tissue radiosensitivity from polymorphisms in candidate genes. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2003; 69:127–35. [PubMed: 14643949]
12. Azria D, Ozsahin M, Kramar A, Peters S, Atencio DP, Crompton NE, et al. Single nucleotide polymorphisms, apoptosis, and the development of severe late adverse effects after radiotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008; 14:6284–8. [PubMed: 18829510]
13. Ambrosone CB, Ahn J, Singh KK, Rezaishiraz H, Furberg H, Sweeney C, et al. Polymorphisms in genes related to oxidative stress (MPO, MnSOD, CAT) and survival after treatment for breast cancer. Cancer research. 2005; 65:1105–11. [PubMed: 15705913]
14. Green H, Ross G, Peacock J, Owen R, Yarnold J, Houlston R. Variation in the manganese superoxide dismutase gene (SOD2) is not a major cause of radiotherapy complications in breast cancer patients. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2002; 63:213–6. [PubMed: 12063011]
15. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med. 1996; 334:1145–9. [PubMed: 8602179]
16. Christen WG, Gaziano JM, Hennekens CH. Design of Physicians' Health Study II--a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. Ann Epidemiol. 2000; 10:125–34. [PubMed: 10691066]
17. Gaziano JM, Glynn RJ, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. JAMA. 2009; 301:52–62. [PubMed: 19066368]
18. Parliament MB. Radiogenomics: associations in all the wrong places? The lancet oncology. 2012; 13:7–8. [PubMed: 22169270]
19. Ioannidis JP. Why most published research findings are false. PLoS medicine. 2005; 2:e124. [PubMed: 16060722]
20. Barnett GC, Coles CE, Elliott RM, Baynes C, Luccarini C, Conroy D, et al. Independent validation of genes and polymorphisms reported to be associated with radiation toxicity: a prospective analysis study. The lancet oncology. 2012; 13:65–77. [PubMed: 22169268]
21. Stark JR, Perner S, Stampfer MJ, Sinnott JA, Finn S, Eisenstein AS, et al. Gleason score and lethal prostate cancer: does 3 + 4 = 4 + 3? Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009; 27:3459–64. [PubMed: 19433685]
22. Bag A, Bag N. Target sequence polymorphism of human manganese superoxide dismutase gene and its association with cancer risk: a review. Cancer epidemiology, biomarkers & prevention : a

publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008; 17:3298–305.

23. Li H, Kantoff PW, Giovannucci E, Leitzmann MF, Gaziano JM, Stampfer MJ, et al. Manganese superoxide dismutase polymorphism, prediagnostic antioxidant status, and risk of clinical significant prostate cancer. Cancer research. 2005; 65:2498–504. [PubMed: 15781667]
24. Kang D, Lee KM, Park SK, Berndt SI, Peters U, Reding D, et al. Functional variant of manganese superoxide dismutase (SOD2 V16A) polymorphism is associated with prostate cancer risk in the prostate, lung, colorectal, and ovarian cancer study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007; 16:1581–6.
25. Chan JM, Oh WK, Xie W, Regan MM, Stampfer MJ, King IB, et al. Plasma selenium, manganese superoxide dismutase, and intermediate- or high-risk prostate cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009; 27:3577–83. [PubMed: 19528373]

## CLINICAL PRACTICE POINTS

Germline polymorphisms in SOD2 may modulate the effect of radiation therapy (RT) by altering local reactive oxygen species. This study examines the predictive impact of germline polymorphisms in SOD2, including the functional rs4880 variant, on lethal prostate cancer after treatment with RT. There was a significant association between SOD2 polymorphisms and lethal prostate cancer. This finding was not validated in a separate cohort with different clinical characteristics but may be specific to a lower-risk population. This study suggests that previous in vitro findings linking SOD2 activity to radiation response may be relevant in the clinical setting as a predictive biomarker of response to RT. The finding remains to be validated in a low-risk cohort.

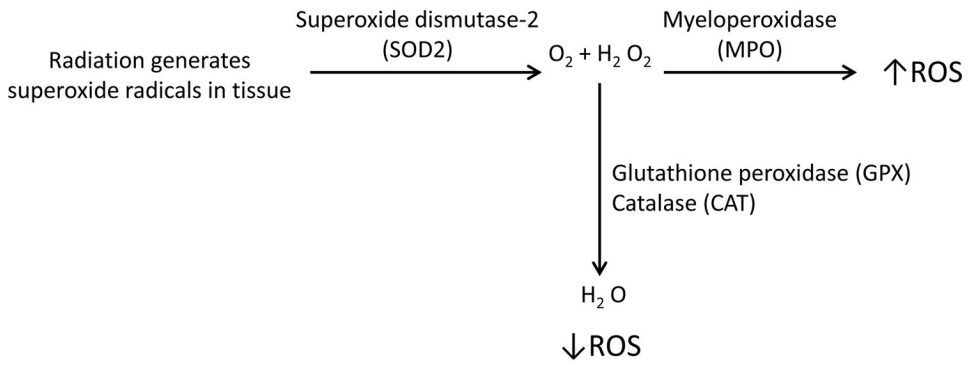


**Figure 1.**

Simplified schema of the relationship between SOD2, reactive oxygen species (ROS), and other enzymes involved in free-radical scavenging in tissue.

## Table 1

Patient characteristics at diagnosis for the test and validation cohorts

| | | Test cohort (n=816) | Validation cohort (n=612) |
|---|---|---|---|
| Follow-up (years), median | | 10.2 | 6.8 |
| Age at treatment (years), median [IQR] | | 73 [68,76] | 64 [59,70] |
| Gleason Score, no. (%) | 2–6 | 486 (60) | 148 (24) |
| | 7 | 196 (24) | 261 (43) |
| | 8–10 | 85 (10) | 173 (28) |
| | Unknown | 49 (6) | 30 (5) |
| Clinical stage, no. (%) | T1/T2 | 744 (91) | 458 (75) |
| | T3/T4/N1 | 50 (6) | 23 (4) |
| | Unknown | 22 (3) | 131 (21) |
| PSA at diagnosis, median [IQR] | | 7.3 ng/mL [5.4,11.0] | 7.7 ng/mL [5.2,15] |
| Year of treatment, no. (%) | 1982–1991 | 83 (10) | 18 (3) |
| | 1992–2001 | 535 (66) | 285 (47) |
| | 2002–2010 | 198 (24) | 309 (51) |

Abbreviations: No. – number; IQR – interquartile range; PSA – prostate-specific antigen

**Table 2**

Allelic variation of the 7 candidate single nucleotide polymorphisms (SNPs) in the *SOD2* gene among study patients in the test (N=816) and validation (N=612) cohorts of men with prostate cancer treated with radiation

| SNP | Major/Minor Allele | MAF (Test) | MAF (Validation) | Type | Annotation |
|---|---|---|---|---|---|
| rs6917589 | (A/G) | 24 % | 24% | - | 3[1] of *SOD2* |
| rs2758331[†] | (G/T) | 48% | 47% | Synonymous | Intron |
| rs4880[†] | (T/C) | 49% | 49% | Non- Synonymous (Valine/Alanine) | Exon2 |
| rs2758329[†] | (A/G) | 48% | 48% | - | 3[1] of *SOD2* |
| rs5746151 | (G/A) | <10% | <10% | - | 3[1] of *SOD2* |
| rs2842980 | (A/T) | 21% | 21% | - | 3[1] of *SOD2* |
| rs7855 | (T/C) | <10% | <10% | - | 3[1] UTR, exon |

Abbreviations: SNP – Single nucleotide polymorphism; MAF – minor allele frequency; A – adenine; G – guanine; T – thymine; C – cytosine; UTR – untranslated region

[†]SNPs are in linkage disequilibrium with each other

**Table 3**

Associations between *SOD2* polymorphisms and lethal prostate cancer among prostate cancer patients undergoing radiation therapy or radical prostatectomy in the test cohort, adjusted for Gleason score, PSA, clinical stage, age at treatment, year of diagnosis and cohort (PHS or HPFS)

| | Radiation Therapy Cohort (N=816) | | | | | | Radical Prostatectomy Cohort (N=1094) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SNP** | **Total** | **Events** | **Unadjusted HR (95% CI)** | **P-value** | **Adjusted HR (95% CI)** | **P-value** | **Total** | **Events** | **Unadjusted HR (95% CI)** | **P-value** | **Adjusted HR (95% CI)** | **P-value** |
| *SOD2* | | | | | | | | | | | | |
| rs6917589 | | | | | | | | | | | | |
| GG | 46 | 5 | 1.70 (0.66, 4.38) | 0.06 | 2.62 (0.98, 7.02) | 0.006 | 39 | 2 | 0.86 (0.21, 3.56) | 0.64 | 0.78 (0.18, 3.25) | 0.54 |
| AG | 230 | 32 | 1.80 (1.10, 2.94) | | 2.21 (1.31, 3.72) | | 334 | 25 | 1.26 (0.75, 2.10) | | 1.31 (0.76, 2.25) | |
| AA | 429 | 32 | REF | | REF | | 598 | 35 | REF | | REF | |
| rs2758331† | | | | | | | | | | | | |
| TT | 177 | 9 | 0.52 (0.24, 1.17) | 0.05 | 0.37 (0.16, 0.83) | 0.04 | 234 | 18 | 1.05 (0.55, 2.01) | 0.66 | 1.10 (0.56, 2.13) | 0.67 |
| GT | 338 | 46 | 1.28 (0.74, 2.20) | | 0.84 (0.47, 1.50) | | 498 | 31 | 0.82 (0.47, 1.46) | | 0.84 (0.47, 1.52) | |
| GG | 193 | 18 | REF | | REF | | 246 | 19 | REF | | REF | |
| rs4880† | | | | | | | | | | | | |
| CC | 182 | 9 | 0.52 (0.23, 1.17) | 0.04 | 0.37 (0.16, 0.84) | 0.04 | 254 | 19 | 1.02 (0.53, 1.94) | 0.77 | 1.10 (0.57, 2.13) | 0.67 |
| TC | 343 | 47 | 1.31 (0.75, 2.28) | | 0.90 (0.50, 1.61) | | 495 | 32 | 0.85 (0.48, 1.51) | | 0.85 (0.47, 1.53) | |
| TT | 182 | 17 | REF | | REF | | 232 | 18 | REF | | REF | |
| rs2758329† | | | | | | | | | | | | |
| GG | 170 | 9 | 0.53 (0.24, 1.18) | 0.05 | 0.39 (0.17, 0.88) | 0.06 | 228 | 18 | 0.99 (0.52, 1.87) | 0.27 | 1.08 (0.56, 2.09) | 0.37 |
| AG | 336 | 46 | 1.27 (0.74, 2.19) | | 0.86 (0.48, 1.53) | | 500 | 27 | 0.66 (0.37, 1.18) | | 0.72 (0.40, 1.31) | |
| AA | 188 | 18 | REF | | REF | | 236 | 20 | REF | | REF | |
| rs5746151 | | | | | | | | | | | | |
| GA/AA | 86 | 7 | 0.75 (0.35, 1.65) | 0.48 | 0.59 (0.25, 1.37) | 0.22 | 121 | 6 | 0.71 (0.31, 1.64) | 0.42 | 0.62 (0.27, 1.46) | 0.27 |
| GG | 627 | 63 | | | REF | | 858 | 61 | REF | | REF | |
| rs2842980 | | | | | | | | | | | | |
| TT | 35 | 2 | 0.59 (0.14, 2.42) | 0.69 | 0.86 (0.20, 3.62) | 0.64 | 43 | 3 | 0.92 (0.29, 2.97) | 0.94 | 0.78 (0.24, 2.53) | 0.79 |
| AT | 224 | 25 | 1.09 (0.67, 1.78) | | 1.26 (0.76, 2.07) | | 327 | 22 | 0.91 (0.55, 1.53) | | 0.85 (0.50, 1.44) | |
| AA | 444 | 46 | REF | | REF | | 604 | 43 | REF | | REF | |
| rs7855 | | | | | | | | | | | | |

| SNP | Radiation Therapy Cohort (N=816) | | | | | | Radical Prostatectomy Cohort (N=1094) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Events | Unadjusted HR (95% CI) | P-value | Adjusted HR (95% CI) | P-value | Total | Events | Unadjusted HR (95% CI) | P-value | Adjusted HR (95% CI) | P-value |
| TC/CC | 75 | 7 | 0.90 (0.41, 1.97) | 0.80 | 0.97 (0.44, 2.13) | 0.94 | 110 | 6 | 0.74 (0.32, 1.71) | 0.48 | 0.45 (0.19, 1.06) | 0.07 |
| TT | 639 | 66 | REF | | REF | | 875 | 64 | REF | | REF | |

Abbreviations: CI – Confidence Interval; SNP – Single nucleotide polymorphism; REF – reference;

[†]SNPs in linkage disequilibrium with each other

**Table 4**

Associations between *SOD2* polymorphisms and distant metastases in the validation cohort (N=612), Dana-Farber Cancer Institute prostate cancer patients who underwent radiation therapy

| SNP | Total | Events | Unadjusted HR (95% CI) | P-value | Adjusted HR (95% CI) | P-value |
|---|---|---|---|---|---|---|
| SOD2 | | | | | | |
| rs6917589 | | | | 0.97 | | 0.97 |
| GG | 42 | 13 | 1.04 (0.59, 1.86) | | 1.04 (0.58, 1.88) | |
| AG | 210 | 51 | 0.97 (0.69, 1.36) | | 1.05 (0.74, 1.48) | |
| AA | 354 | 100 | REF | | REF | |
| rs2758331 | | | | 0.99 | | 0.85 |
| TT | 128 | 36 | 1.02 (0.66, 1.57) | | 0.99 (0.63, 1.55) | |
| GT | 296 | 82 | 1.03 (0.72, 1.48) | | 1.09 (0.76, 1.58) | |
| GG | 180 | 46 | REF | | REF | |
| rs4880 | | | | 0.94 | | 0.87 |
| CC | 141 | 38 | 0.93 (0.60, 1.44) | | 0.93 (0.60, 1.46) | |
| TC | 298 | 82 | 0.98 (0.68, 1.42) | | 1.04 (0.71, 1.52) | |
| TT | 165 | 44 | REF | | REF | |
| rs2758329 | | | | 0.99 | | 0.90 |
| GG | 136 | 37 | 0.97 (0.63, 1.50) | | 0.94 (0.60, 1.46) | |
| AG | 296 | 82 | 1.00 (0.70, 1.44) | | 1.03 (0.71, 1.50) | |
| AA | 173 | 45 | REF | | REF | |
| rs5746151 | | | | 0.18 | | 0.14 |
| GA/AA | 78 | 25 | 1.34 (0.87, 2.06) | | 1.40 (0.89, 2.20) | |
| GG | 528 | 139 | REF | | REF | |
| rs2842980 | | | | 0.54 | | 0.33 |
| AT/TT | 238 | 63 | 0.91 (0.66, 1.24) | | 0.85 (0.61, 1.18) | |
| AA | 367 | 101 | REF | | REF | |
| rs7855 | | | | 0.63 | | 0.14 |
| TC/CC | 72 | 19 | 0.89 (0.55, 1.44) | | 0.69 (0.41, 1.14) | |
| TT | 534 | 145 | REF | | REF | |

Abbreviations: SNP – Single nucleotide polymorphism; HR – Hazard ratio; CI – Confidence Interval; REF – reference