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## Analysis of Single Nucleotide Polymorphisms and Radiation Sensitivity of the Lung Assessed With an Objective Radiologic Endpoint

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### Abstract

To explore the possibility that underlying genetic susceptibility influences radiation-induced lung injury, we studied pulmonary damage after radiation therapy for lung cancer and single nucleotide polymorphisms implicated in radiation sensitivity. An objective radiologic method (perfusion single photon emission computed tomography) was used to assess radiation sensitivity. An association between single nucleotide polymorphisms in XRCC1 and BRCA1 and radiation sensitivity of the lungs was noted.

**Background**—The primary objective of this study was to evaluate the association between radiation sensitivity of the lungs and candidate single nucleotide polymorphisms (SNP) in genes implicated in radiation-induced toxicity.

**Methods**—Patients with lung cancer who received radiation therapy (RT) had pre-RT and serial post-RT single photon emission computed tomography (SPECT) lung perfusion scans. RT-induced changes in regional perfusion were related to regional dose, which generated patient-specific dose-response curves (DRC). The slope of the DRC is independent of total dose and the irradiated volume, and is taken as a reflection of the patient's inherent sensitivity to RT. DNA was extracted from blood samples obtained at baseline. SNPs were determined by using a combination of high-resolution melting, TaqMan assays, and direct sequencing. Genotypes from 33 SNPs in 22 genes were compared against the slope of the DRC by using the Kruskal-Wallis test for ordered alternatives.

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#### Disclosure

The authors have stated that they have no conflicts of interest.

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**Results**—Thirty-nine self-reported Caucasian patients with pre-RT and 6 month post-RT SPECTs, and blood samples were identified. An association between genotype and increasing slope of the DRC was noted in G(1301) A in *XRCC1* (rs25487) ( $P = .01$ ) and G(3748) A in *BRCA1* (rs16942) ( $P = .03$ ).

**Conclusions**—By using an objective radiologic assessment, polymorphisms within genes involved in repair of DNA damage (*XRCC1* and *BRCA1*) were associated with radiation sensitivity of the lungs.

### Keywords

Genetics; Lung toxicity; Radiation sensitivity; Single photon emission computed tomography; Single-nucleotide polymorphism

### Introduction

Radiation therapy (RT) is an important treatment modality for patients with cancer, and approximately 50% to 60% of cancer patients will receive RT at some point during their illness. The adverse effects of RT depend principally on the site being treated, the volume of normal tissue irradiated, the dose per fraction, the total dose administered, and whether chemotherapy is also being administered. However, it is also clinically apparent that some cancer patients are more sensitive to the adverse effects of RT than are others, even when all of the factors listed above are relatively constant. Rare hereditary disorders (eg, ataxia-telangiectasia, Nijmegen Breakage syndrome, Fanconi anemia) suggest that genetic differences in key genes may influence radiation sensitivity.<sup>1</sup>

Single nucleotide polymorphisms (SNPs) are DNA sequence variations, in either coding or noncoding regions, in which a single nucleotide (adenine, guanine, thymine, and cytosine) varies among a population. To identify genetic factors that may be contributing to radiation-induced toxicity, SNPs in genes associated with DNA repair pathways and other radiation-related processes have been studied. By using this candidate gene approach, some studies, but not all,<sup>2–4</sup> have demonstrated an association between certain SNPs and acute and long-term adverse effects of RT. These genes include *TGFBI*,<sup>5–11</sup> *SOD2*,<sup>12,13</sup> *XRCC1*,<sup>11–14</sup> *XRCC3*,<sup>13,15,16</sup> *XRCC6*,<sup>16</sup> *MSH2*,<sup>15</sup> *MSH3*,<sup>15</sup> *ATM*,<sup>17,18</sup> *p53*,<sup>19</sup> *FSHR*,<sup>20</sup> *ABCA1*,<sup>21</sup> *IL12RB2*,<sup>21</sup> *LIG4*,<sup>22</sup> and *RAD51*.<sup>23</sup>

Most of the endpoints studied to date have been subjective signs or symptoms reported by the patient or provider (eg, erectile dysfunction or breast fibrosis). More objective outcomes may be preferred when evaluating potential genetic contributors of radiation sensitivity. As part of institutional review board–approved prospective studies at Duke University, patients with lung cancer have undergone serial perfusion single photon emission computed tomographies (SPECT) before and after thoracic RT. Dose-dependent changes in SPECT lung perfusion defects after treatment provide an objective assessment of inherent radiation sensitivity.<sup>24,25</sup> Most of these same patients had blood samples collected and stored for correlative studies.

We recently reported an association between a polymorphism in the promoter of the transforming growth factor  $\beta 1$  gene (*TGFBI*) and radiation sensitivity assessed by using radiation-induced SPECT changes.<sup>26</sup> Herein, we assess for the possible association between 12 additional SNPs that have been associated with radiation sensitivity in previous studies, in addition to numerous other SNPs within genes known to be involved in DNA damage recognition and repair, which may contribute to radiation sensitivity.

## Materials and Methods

### Patient Population

As part of institutional review board–approved prospective clinical trials at Duke University, patients with lung cancer who were receiving definitive RT underwent a pretreatment perfusion SPECT scan as well as serial posttreatment scans to assess radiation-induced lung injury. Blood was drawn on several patients at baseline for correlative studies and was stored at  $-80^{\circ}\text{C}$ . Patients were included in the present analysis if they (1) underwent a pre-RT SPECT, (2) underwent a 6-month post-RT SPECT, (3) had at least 1 banked blood sample, and (4) were Caucasian. Changes in SPECT perfusion after RT largely develop within the first 6 months after treatment with minimal changes thereafter.<sup>25,27</sup> Only self-reported Caucasian patients were studied because the relative allelic frequency of SNPs may differ among ancestries.

### RT Planning

Patients underwent computed tomography (CT) based 3-dimensional treatment planning by using Plan University of North Carolina software (PLUNC). The patients either received conventionally fractionated RT (1.8–2 Gy every day to 40–70 Gy) or accelerated RT by using a concomitant boost.<sup>28</sup> For the latter, the patients received 1.25 Gy twice a day to the clinical target volume, including the primary tumor and mediastinum, usually with anteroposterior/posteroanterior fields. The gross tumor volume received a concurrent boost of 35 cGy twice a day with off-cord fields, thus delivering 1.6 Gy twice a day to the gross tumor volume. After the initial 57.6 Gy, the gross tumor volume received an additional dose at 1.6 Gy twice a day to a total dose of 73.6–86.4 Gy). Chemotherapy was administered at the discretion of the multimodality team.

### Perfusion SPECTs

SPECT lung perfusion scans were obtained after the intravenous injection of technetium-99m–labeled macroaggregated albumin as previously described.<sup>24,29,30</sup> The pre- and post-radiation SPECT lung images were registered to each other and to the radiation treatment planning scan (and hence the 3-dimensional dose distribution), largely manually with the assistance of some automatic image registration tools. The SPECT images were translated and rotated (in 6 degrees of freedom) until the “edges” of the SPECT-defined perfusion (the area of rapid gradient in counts per cubic centimeter) were aligned with the CT-defined lung borders, also considering the presence of the tumor and regions of emphysema that influence the SPECT images.<sup>31</sup> We recognize that a perfect registration is not possible for an elastic organ in a breathing patient. Every attempt was made to have all of the scans and the radiation treatment delivered with the patient in a similar position. In all cases, the registration was performed by an experienced physicist.

This multi-image registration facilitated the analysis relating changes in regional perfusion (comparison of pre- and post-radiation SPECTs) to the regional radiation dose (from the planning CT). After registration, the quantitative SPECT data were resampled by tri-linear interpolation to match the spatial sampling of the planning CT data set. Within each lung pixel, the change in regional perfusion was quantified by comparing pre- and post-radiation SPECTs.<sup>27</sup> For each patient, and at each dose level ( $D$ ), the reduction in the percentage of SPECT counts (compared with the pre-RT scan) was calculated as percent reduction $_D = 100 \times (1 - \text{post}_D) / \text{pre}_D$ ; in which  $\text{post}_D$  and  $\text{pre}_D$  are the percentage SPECT counts on the post- and pre-RT scans in the region that received  $D$  Gy, respectively.<sup>27</sup>

Changes in regional perfusion after RT were related to regional radiation doses (via image fusion), which yielded a patient-specific dose-response curve (DRC). The DRC slope is

independent of irradiated volume and is taken as a reflection of the patient's inherent sensitivity to radiation. The DRC was obtained from the nontumor-bearing contralateral lung to avoid issues related to reperfusion after treatment of central tumors. Because SPECTs provide only relative perfusion information, the DRC was "normalized" by assuming that absolute perfusion is unchanged in "control" regions that receive very low doses (typically <2.5 Gy).<sup>27</sup> The normalized percent reduction in regional perfusion,  $R$ , at dose  $D$ , is thus<sup>32</sup>:

$$R_D = 100 \times [1 - (\text{post}_D / \text{post}_0 \div \text{pre}_D / \text{pre}_0)].$$

In which the 0 subscripts refer to the lung regions at <2.5 Gy. We recognize that normalization may be imprecise because function in the low-dose region may increase due to compensation. Nevertheless, this is the method that has been used in the 2 centers that have generated DRCs, and we know of no way to correct for this possibility.

### Studied SNPs

Genes of interest were selected based on peer-reviewed publications and knowledge of DNA damage repair pathways. Each gene was reviewed on the National Center for Biotechnology Information database. SNPs within genes with a minor allele frequency more than 0.10 in a Caucasian population were selected for analysis. SNPs within coding regions that resulted in a synonymous mutation (no change in amino acid) were not included. Hardy-Weinberg equilibrium was assessed for all SNPs.

### Genotyping Methods

DNA extraction was performed by using the QIAamp DNA Blood Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. DNA was found to be of high quality, and no samples had to be discarded secondary to impurity or degradation. SNP genotypes were determined by using a combination of high-resolution melting (HRM) assays, TaqMan assays, and direct DNA sequencing. Primer pairs for each SNP were designed by using the National Center for Biotechnology Information-Primer Blast program to create approximately 125 bp amplicons that contain each SNP. HRM assays were performed on an Applied Biosystems 7500 Fast Real-Time polymerase chain reaction system according to the manufacturer's instructions and were analyzed with Applied Biosystems HRM v2.0 software (Applied Biosystems, Foster, CA). Based on the HRM curves, selected samples were DNA sequenced to confirm the SNP genotype by using the Duke University DNA Analysis Facility shared resource. Predefined or custom-designed TaqMan SNP genotyping assays were obtained from Applied Biosystems and were performed on a 7500 Fast Real-Time polymerase chain reaction instrument according to the manufacturer's standard protocol by using 20-ng human genomic DNA per 20  $\mu$ L assay. Data analysis was performed by using Applied Biosystems 7500 Software v2.0.4.

### Statistical Analysis

All statistical summaries and analyses were produced by using the R statistical environment.<sup>33</sup> The SNPs were tested for the Hardy-Weinberg equilibrium by using an exact test<sup>34</sup> provided by the R extension package genetics. SNPs not in Hardy-Weinberg equilibrium were not further assessed. The association between genotype and the slope of the DRC was tested for each SNP by using the Kruskal-Wallis equilibrium powered for ordered alternatives.<sup>35</sup> The implementation provided by the R extension package coin<sup>36</sup> was used for this purpose. The analyses for all SNPs in this study were a priori powered for additive genetic effects. Because this was an exploratory study, the results were not adjusted for multiple testing to minimize the risk of false negatives.

## Results

### Patient Characteristics

Forty-five unrelated patients with a baseline blood sample and pre-RT and 6-month post-RT SPECTs, and with usable DNA, were identified. Patients who did not self-report as Caucasian were excluded ( $n = 6$ ), which left 39 for the present analysis. The majority had a history of smoking (92%). Patients received either conventionally fractionated RT ( $n = 29$ ) or accelerated hyperfractionated RT ( $n = 10$ ), with chemotherapy administered in the majority (82%). Chemotherapy consisted of a platinum-based doublet in all patients (96% carboplatin and 4% cisplatin). The median total radiation dose was 66 Gy (range, 40–86.4 Gy). Patient and treatment characteristics can be found in Table 1.

All the patients underwent pre- and post-radiation SPECTs. The median time between completing treatment and follow-up SPECT was 8 months (range, 6–17 months). The median DRC slope was 0.38 (ie, 0.38% reduction in perfusion per Gy), with a range of  $-1.14$  to  $1.12$ . There was no correlation between DRC slope and the time interval of follow-up SPECT, consistent with our prior analyses for time intervals  $>6$  months.<sup>25,27</sup>

Forty-three SNPs in 23 genes were initially screened. Eight SNPs had a relative minor allele frequency of zero and were not evaluated further (rs28897686, rs28897687, and rs28897688 in *BRCA1*; rs2229033 in *ATR*; rs3730017 in *NOS2*; rs75521089 in *HIF1A*; rs28897729 in *BRCA2*; and rs80233386 in *RAD51*). Genotyping results from 1 SNP (rs1650697 in *MSH3*) were not thought to be reliable, and the results were discarded. One SNP was not in Hardy-Weinberg equilibrium and was not assessed further (rs1801321 in *RAD51*). This left 33 SNPs in 22 genes for the present analysis (Table 2).

Among the 33 SNPs studied, 2 were associated with increasing slope of the SPECT DRC (Table 3). These included rs16942 (a SNP in exon 10 of *BRCA1*) ( $P = .03$ ) and rs25487 (a SNP in exon 10 of *XRCC1*) ( $P = .01$ ) (Figure 1). None of the other studied SNPs were statistically significant (Table 3). As both *XRCC1* and *BRCA1* are directly involved or regulate base excision repair, a simple parametric linear SNP-SNP association analysis, with both SNPs coded additively, was performed. The asymptotic  $P$  value for the interaction was .67. Due to the small sample size, the resulting sparseness in the data may render this analysis of limited use.

## Discussion

By using this unique database, an objective radiologic endpoint was available to assess radiation sensitivity of the lungs. Radiographic abnormalities, indicative of injury, are noted in the lung almost immediately after thoracic RT.<sup>27,37</sup> The radiographic findings most extensively studied are increased density on CT and decreased pulmonary perfusion on SPECT. Radiation dose is the single most important contributor to the development of radiographic abnormalities.<sup>38,39</sup> An optimal endpoint to study radiation sensitivity would be both objective and clinically relevant. SPECT abnormalities, admittedly, are only weakly correlated with clinical outcomes, such as change in pulmonary function or development of symptoms.<sup>40</sup> However, radiation pneumonitis, which has also been studied in the context of SNPs,<sup>5,14,19,22,23,41–43</sup> is notoriously difficult to accurately identify and grade because it is a clinical diagnosis.<sup>44</sup> Further, although clinical symptoms are dependent on dosimetric parameters (the volume of lung that receives a certain radiation dose), the SPECT DRC is independent of volume. Thus, both approaches should be viewed as complementary when studying genetic susceptibility to RT.

In this study, when using the slope of the SPECT DRC, we observed that 2 single nucleotide polymorphisms, one in *XRCC1* and one in *BRCA1*, were associated with radiation sensitivity of the lungs assessed with SPECT. In a prior analysis, the -509 promoter in the *TGFBI* gene was also associated with radiation sensitivity.<sup>26</sup> Multiple other studies have also observed an association among the -509 SNP in *TGFBI* and radiation toxicities, including altered breast appearance,<sup>7</sup> breast fibrosis,<sup>8,9</sup> erectile dysfunction,<sup>6</sup> rectal bleeding, and miscellaneous severe complications.<sup>10</sup> However, several other studies have not observed such relationships.<sup>2,45,46</sup>

The protein product of *XRCC1* is involved in the base excision repair pathway in which single-strand breaks, generated after exposure to ionizing radiation or other harmful stimuli, are repaired. An association between radiation sensitivity and an SNP in exon 10 of *XRCC1* was observed in our study (rs25487). This polymorphism substitutes an A (adenine) for G (guanine) at messenger RNA position 1316, which leads to an amino acid substitution at position 399 (glutamine for arginine). Patients with the ancestral allele (G) were found to be more radiosensitive.

Yin et al<sup>14</sup> observed that the *XRCC1* Q399R AA genotype, after correcting for potential confounding factors, was associated with a reduced risk of radiation pneumonitis in patients with non-small-cell lung cancer (0.48) ( $P = .04$ ). Andreassen et al<sup>13</sup> observed an association between G/G homozygotes and radiation-induced subcutaneous fibrosis after treatment for breast cancer, with heterozygotes at intermediate risk compared with AA homozygotes. Similarly, in a study of patients with nasopharyngeal cancer, Alsbeih et al<sup>11</sup> noted an association between the G allele and grade 2 to 3 late fibrosis after head-and-neck RT for nasopharyngeal carcinoma. Chang-Claude et al<sup>47</sup> observed a trend for a higher risk of acute adverse effects in breast cancer patients with normal weight and with this same genetic change, although the difference was not statistically significant.

However, Giotopoulos et al<sup>9</sup> studied patients with breast cancer who received either postlumpectomy or postmastectomy RT (without an electron boost) and evaluated high-grade telangiectasias and fibrosis. SOMA score 2 to 4 telangiectasias developed in 2% of patients with GG, 11% of patients with AG, and 5% of patients with AA. It was reported that heterozygosity was more likely to develop telangiectasias ( $P = .01$ ). Because AA homozygotes had a lower risk than heterozygotes, the biologic significance of this is ambiguous. Burri et al<sup>12</sup> did not observe an association between this polymorphism and complications after brachytherapy, with or without external-beam RT (rectal bleeding, erectile dysfunction, urinary morbidity). Other investigators have also not found an association between this polymorphism and subjective endpoints of radiation sensitivity, including breast fibrosis,<sup>48</sup> altered breast appearance after RT,<sup>2,7</sup> and acute skin reactions.<sup>49</sup>

A pooled analysis of several studies (differing endpoints, scoring systems, patient populations) by Langsenlehner et al<sup>50</sup> did not observe an effect of the *XRCC1* R399Q polymorphism on risk of late radiation-induced toxicity. There was an association with *XRCC1* R280H, with an odds ratio of 0.6. We did not observe an association with this polymorphism ( $P = .98$ ), but there were only 4 heterozygotes in our population.

This is the first study, to our knowledge, that has demonstrated a possible association between the G(3780)A SNP in *BRCA1* and radiation sensitivity of the lungs. *BRCA1*, typically associated with inherited breast cancer, encodes a protein that joins other proteins involved in DNA damage recognition and repair to form a complex known as the BRCA1-associated genome surveillance complex. *BRCA1* plays a central role in repairing DNA double-strand breaks. A lower risk of toxicity was noted in patients with the AG or GG genotype. Few studies have evaluated BRCA1 polymorphisms and their possible

relationship to radiation toxicity. Damaraju et al<sup>51</sup> did not observe an association among any *BRCA1* SNPs, including position 3780, and bladder and/or rectal toxicity after pelvic RT. We did not observe an association among many SNPs that had previously been associated with RT toxicity (Table 4). This may be due to a lack of statistical power, lack of true causation, or organ and/or tissue specific differences.

To date, most studies that evaluated radiation toxicity have used relatively subjective endpoints. This is understandable because most toxicities that patients experience are not readily quantifiable. Our study was unique because we used an objective measure of RT sensitivity, namely dose-dependent changes in regional perfusion in the lung after thoracic RT. We have observed possible associations between SNPs in *TGFβ1*, *XRCC1*, and *BRCA1*, and a greater decline in pulmonary perfusion after RT. Large, genome-wide association studies may ultimately be required to discover which polymorphisms are most responsible for interpatient differences in radiation sensitivity. Ideally, objective and clinically relevant endpoints of radiation sensitivity will be used in these endeavors.

We acknowledge the limitations of our analysis. The number of patients is small, and a verification cohort is not available. We were constrained to use a candidate gene approach as opposed to a genome-wide association study, given the small number of patients with available SPECT data to perform our analysis. We also did not correct for multiple hypotheses given the small number of patients. Thus, the associations observed could have been due to chance alone. Finally, multivariate analyses to correct for possible confounding factors (chemotherapy, smoking status, pulmonary function, etc) were not deemed statistically feasible in our population. These same factors did not have a dramatic effect on the DRC in our prior analyses.<sup>39</sup> The fundamental and unique strength of our study, as opposed to almost all other studies that evaluated radiation toxicity, is the objective radiologic endpoint of radiation sensitivity that was used. In this study, in which an objective radiologic assessment of radiation-induced lung injury was used, polymorphisms within genes involved in repair of DNA damage (*XRCC1* and *BRCA1*) were associated with radiation sensitivity of the lungs.

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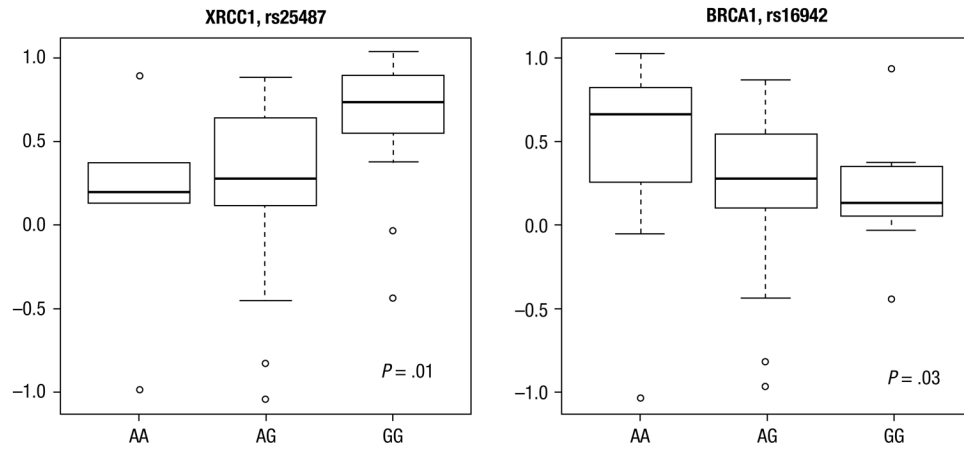


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### Clinical Practice Points

- Dosimetric factors predict the risk of radiation-induced lung injury but have limitations.
- Underlying genetic factors, including SNPs, may contribute to risk.
- Further work is necessary to evaluate whether genetic factors are contributory and how this should change clinical treatment recommendations.



**Figure 1.**

Boxplots for rs25487 (XRCC1) and rs16942 (BRCA1), Produced by Using the R Boxplot Function With Its Default Settings, Comparing Genotype With Slope of the Single Photon Emission Computed Tomography Dose-Response Curve. The Line in the Middle Is the Median (Q2). The Upper and Lower Edges of the Box Are the 25th (Q1) and 75th (Q3) Percentiles. The Height of the Box is the Interquartile Range (IQR). The Whiskers Are Computed as Follows: Exclude All Data Points That Are Either  $1.5 \times \text{IQR}$  Below Q1 or  $1.5 \times \text{IQR}$  Above Q3. The Whiskers Correspond to the Range of This Reduced Data Set. Any Data Points (outliers) From the Original Sample Below or Above the Whiskers Are Also Plotted

**Table 1**

Patient Characteristics (n = 39)

Characteristic	
<b>Median Age (Range), y</b>	63 (46–87)
<b>Sex, No. (%)</b>	
Men	20 (51)
Women	19 (49)
<b>Stage, No. (%)</b>	
I	3 (8)
II	2 (5)
III	27 (69)
IV	3 (8)
X (locally)	4 (10)
<b>Histology, No. (%)</b>	
Squamous	5 (13)
Adenocarcinoma	6 (15)
Non-small-cell not otherwise specified	24 (62)
Small cell	4 (10)
<b>Smoking Status, No. (%)</b>	
Current	12 (31)
Former	24 (61)
Never	3 (8)
<b>Treatment, no. (%)</b>	
Sequential ChT/RT	22 (56)
Concurrent ChT/RT <sup>a</sup>	10 (26)
RT alone	7 (18)
<b>Median Radiation Dose (range), Gy</b>	66 (40–86.4)
<b>Mean Lung Dose, Median (range), Gy</b>	18.5 (5–29)
<b>Median V<sub>20</sub> (range)</b>	33 (6–51)

Abbreviations: ChT = chemotherapy; RT = radiation therapy; V<sub>20</sub> = volume of lung receiving 20 Gy or more.

<sup>a</sup>With or without induction and/or consolidation ChT.

Table 2

Genes and SNPs Studied

SNP	Gene	Location	Position	Base Substitution	Amino Acid Position	Amino Acid Substitution
rs2071746	HMOX1	Promoter	-413	A → T	NA	NA
rs2071747	HMOX1	Exon 1	99	G → C	7	Asp → His
rs2779249	NOS2A	Promoter	974	C → A	NA	NA
rs2297518	NOS2A	Exon 16	2087	G → A	608	Ser → Leu
rs11549465	HIF1A	Exon 12	2148	C → T	582	Pro → Ser
rs1801320	RAD51	Promoter	-135	G → C	NA	NA
rs11226	RAD52	Promoter	-2259	C → T	NA	NA
rs1042522	TP53	Exon 4	412	C → G	72	Pro → Arg
rs1805388	LIG4	Exon 2	261	C → T	9	Thr → Ile
rs1062557	GRP	Exon 1	108	C → A	4	Arg → Ser
rs1801516	ATM	Exon 37	5942	G → A	1853	Asp → Asn
rs16941	BRCA1	Exon 10	3345	A → G	1038	Glu → Gly
rs16942	BRCA1	Exon 10	3780	G → A	1183	Arg → Lys
rs144848	BRCA2	Exon 10	1341	T → G	372	Asn → His
rs1799782	XRCC1	Exon 6	700	C → T	194	Arg → Trp
rs25489	XRCC1	Exon 9	959	G → A	280	Arg → His
rs25487	XRCC1	Exon 10	1316	G → A	399	Arg → Gln
rs3218536	XRCC2	Exon 3	649	G → A	188	Arg → His
rs861539	XRCC3	Unknown	1045	C → T	241	Thr → Met
rs4880	SOD2	Unknown	201	T → C	16	Val → Ala
rs13181	ERCC2	Exon 23	2298	G → T	751	Lys → Gln
rs2227928	ATR	Exon 4	754	C → T	211	Thr → Met
rs2229032	ATR	Exon 43	7396	G → A	2425	Arg → Gln
rs1130409	APEX1	Exon 5	776	G → T	148	Glu → Asp
rs26279	MSH3	Unknown	3386	G → A	1045	Ala → Thr
rs184967	MSH3	Unknown	3099	G → A	949	Arg → Gln
rs1799977	MLH1	Unknown	715	A → G	219	Ile → Val

SNP	Gene	Location	Position	Base Substitution	Amino Acid Position	Amino Acid Substitution
rs3790566	IL12RB2	Intron	NA	T → C	NA	NA
rs3790567	IL12RB2	Intron	NA	A → G	NA	NA
rs3790568	IL12RB2	Intron	NA	G → A	NA	NA
rs2230806	ABCA1	Unknown	969	G → A	219	Arg → Lys
rs2253304	ABCA1	Intron	NA	A → G	NA	NA
rs2487058	ABCA1	Intron	NA	C → T	NA	NA

Abbreviations: A = adenine; C = cytosine; G = guanine; NA = not applicable; SNP = single nucleotide polymorphism; T = thymine.

**Table 3**Genotype Frequencies and Association With Slope of DRC<sup>a</sup>

SNP	Gene	Genotypes	P Value
rs2071746	HMOX1	9 AA, 22 AT, 8 TT	.59
rs2071747	HMOX1	36 GG, 3 GC	.67
rs2779249	NOS2A	4 AA, 23 AC, 12 CC	.54
rs2297518	NOS2A	3 AA, 14 AG, 22 GG	.73
rs11549465	HIF1A	27 CC, 11 CT, 1 TT	.52
rs1801320	RAD51	34 GG, 5 CG	.21
rs11226	RAD52	14 CC, 18 CT, 7 TT	.21
rs1042522	TP53	22 GG, 17 GC	.97
rs1805388	LIG4	25 CC, 13 CT, 1 TT	.93
rs1062557	GRP	25 AA, 12 AC, 2 CC	.18
rs1801516	ATM	1 AA, 11 AG, 27 GG	.58
rs16941	BRCA1	19 AA, 15 AG, 5 GG	.47
rs16942	<b>BRCA1</b>	<b>19 AA, 13 AG, 7 GG</b>	<b>.03</b>
rs144848	BRCA2	2 GG, 15, GT, 22 TT	.09
rs1799782	XRCC1	33 CC, 6 CT	.24
rs25489	XRCC1	35 GG, 4 AG	.98
rs25487	<b>XRCC1</b>	<b>6 AA, 20 AG, 13 GG</b>	<b>.01</b>
rs3218536	XRCC2	1 AA, 6 AG, 32 GG	.83
rs861539	XRCC3	14 CC, 18 CT, 7 TT	.09
rs4880	SOD2	11 CC, 16 CT, 12 TT	.98
rs13181	ERCC2	3 GG, 23 GT, 13 TT	.66
rs2227928	ATR	15 CC, 19 CT, 5 TT	.44
rs2229032	ATR	29 GG, 10 GA	.16
rs1130409	APEX1	12 GG, 21 GT, 6 TT	.27
rs26279	MSH3	17 AA, 19 AG, 3 GG	.20
rs184967	MSH3	29 GG, 9 GA, 1 AA	.30
rs1799977	MLH1	12 AA, 23 AG, 4 GG	.15
rs3790566	IL12RB2	22 CC, 17 CT	.33
rs3790567	IL12RB2	22GG, 17 GA	.32
rs3790568	IL12RB2	33 GG, 6 GA	.89
rs2230806	ABCA1	13 CC, 21 CT, 5 TT	.60
rs2253304	ABCA1	12 GG, 21 GA, 6 AA	.97
rs2487058	ABCA1	15 CC, 19 CT, 5 TT	.98

Bold indicates statistically significant associations.

Abbreviations: A = adenine; C = cytosine; DRC = dose-response curve; G = guanine; SNP = single nucleotide polymorphism.

<sup>a</sup>DRC of perfusion single photon emission computed tomography after radiation therapy.



**Table 4**

## SNPs Associated With Radiation Sensitivity

Gene	Name	SNP	Endpoint <sup>a</sup>
<b>TGFB1</b>	Transforming growth factor $\beta$ 1	rs1800469; rs1800470; rs1800471	Radiation pneumonitis, <sup>5</sup> altered breast appearance, <sup>7</sup> breast fibrosis, <sup>7,8</sup> erectile dysfunction, <sup>6</sup> rectal bleeding, <sup>6</sup> miscellaneous severe complications, <sup>10</sup> neck fibrosis, <sup>11</sup> perfusion SPECT defects <sup>26</sup>
<b>SOD2</b>	Superoxide dismutase 2	rs4880	Rectal bleeding, <sup>12</sup> subcutaneous fibrosis <sup>13</sup>
<b>XRCC1</b>	X-ray repair cross- complementing protein 1	rs25489; rs25487	Erectile dysfunction, <sup>12</sup> subcutaneous fibrosis, <sup>11,13</sup> rectal/bladder toxicity, <sup>50</sup> pneumonitis <sup>14</sup>
<b>XRCC3</b>	X-ray repair cross- complementing protein 3	rs861539	Acute skin reaction, <sup>15</sup> subcutaneous fibrosis and telangiectasia, <sup>13</sup> severe dysphagia <sup>16</sup>
<b>MSH2</b>	mutS homolog 2	rs2303428	Acute skin reaction <sup>15</sup>
<b>MSH3</b>	mutS homolog 3	rs26279	Acute skin reaction <sup>15</sup>
<b>ATM</b>	Ataxia telangiectasia mutated	Multiple	Rectal bleeding, <sup>17</sup> erectile dysfunction, <sup>17</sup> urinary morbidity, <sup>17</sup> chest wall fibrosis, <sup>18</sup> subcutaneous late effects, <sup>52</sup> pneumonitis, <sup>43</sup> miscellaneous adverse reactions, <sup>53</sup> late effects <sup>54</sup>
<b>MC1R</b>	Melanocortin 1 receptor	Multiple	Severe acute adverse effects <sup>55</sup>
<b>FSHR</b>	Follicle-stimulating hormone receptor	rs2268363	Erectile dysfunction <sup>20</sup>
<b>P53</b>	Tumor protein p53	rs1042522	Radiation pneumonitis <sup>19</sup>
<b>ABCA1</b>	ATP-binding cassette, subfamily A, member 1	rs2230806; rs2253304; rs2487058	Radiation dermatitis <sup>21</sup>
<b>IL12RB2</b>	Interleukin-12 receptor, beta 2	rs379056; rs3790566; rs3790568	Radiation dermatitis <sup>21</sup>
<b>RAD51</b>	RAD51 homolog	rs1801320	Pneumonitis <sup>23</sup>
<b>LIG4</b>	Ligase IV, DNA, ATP-dependent	rs1805388	Pneumonitis <sup>22</sup>

Abbreviations: ATP = adenosine triphosphate; SNP = single nucleotide polymorphism; SPECT = single photon emission computed tomography.

<sup>a</sup>Not all SNPs are associated with all endpoints.