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Biology Contribution

SNP in *TXNRD2* Associated With Radiation-Induced Fibrosis: A Study of Genetic Variation in Reactive Oxygen Species Metabolism and Signaling

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Summary

In a 2-stage study, we identified an association between level of radiation-induced subcutaneous fibrosis and a nonsynonymous single-nucleotide polymorphism (SNP) in *TXNRD2* (rs1139793) in a cohort of 92 breast cancer (BC) survivors. The association was confirmed in an independent cohort of 283 BC survivors.

Purpose: The aim of the study was to identify noninvasive markers of treatment-induced side effects. Reactive oxygen species (ROS) are generated after irradiation, and genetic variation in genes related to ROS metabolism might influence the level of radiation-induced adverse effects (AEs).

Methods and Materials: 92 breast cancer (BC) survivors previously treated with hypofractionated radiation therapy were assessed for the AEs subcutaneous atrophy and fibrosis, costal fractures, lung fibrosis, pleural thickening, and telangiectasias (median follow-up time 17.1 years). Single-nucleotide polymorphisms (SNPs) in 203 genes were analyzed for association to AE grade. SNPs associated with subcutaneous fibrosis were validated in an independent BC survivor material (n = 283). The influence of the studied genetic variation on messenger ribonucleic acid (mRNA) expression level of 18 genes previously associated with fibrosis was assessed in fibroblast cell lines from BC patients.

Results: Subcutaneous fibrosis and atrophy had the highest correlation ($r = 0.76$) of all assessed AEs. The nonsynonymous SNP rs1139793 in *TXNRD2* was associated with grade of subcutaneous fibrosis, the reference T-allele being more prevalent in the group experiencing severe

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Hege Ladmark-Høyvik and Kristin Valborg Reinertsen are both 2nd authors.

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TXNRD2 is a mitochondrial enzyme not previously implicated in the etiology of fibrosis. The enzyme is a central player in ROS scavenging and is linked to the mitochondrial respiratory chain.

levels of fibrosis. This was confirmed in another sample cohort of 283 BC survivors, and rs1139793 was found significantly associated with mRNA expression level of *TXNRD2* in blood. Genetic variation in 24 ROS-related genes, including *EGFR*, *CENPE*, *APEX1,* and *GSTP1,* was associated with mRNA expression of 14 genes previously linked to fibrosis ($P \leq .005$).

Conclusion: Development of subcutaneous fibrosis can be associated with genetic variation in the mitochondrial enzyme TXNRD2, critically involved in removal of ROS, and maintenance of the intracellular redox balance.

Introduction

Earlier detection and more effective multimodal treatments have improved the prognosis for many cancers. Consequently, the number of cancer survivors is growing, and the importance of identifying factors influencing the level of treatment-induced adverse effects (AEs) increases.

Ionizing radiation (IR) is an important cancer treatment in both a curative and a palliative setting. Like most cancer treatments, radiation therapy has the power to heal but is also associated with long-term complications, both dependent on the properties of the administered radiation therapy and on the irradiated tissue (1). In breast cancer (BC) survivors, such long-term sequelae include lymphedema, impaired shoulder mobility, cardiac dysfunction, costal fractures, pleural thickening, and subcutaneous fibrosis (2-4).

The effects of IR result from energy deposition in the irradiated tissues (5). IR may directly damage cellular macromolecules, including deoxyribonucleic acid (DNA). The indirect IR effects are mediated through the generation of reactive oxygen species (ROS) and subsequent by-products, which may ultimately damage DNA, RNA, and proteins, and deregulate transcription factors and kinases (5). Several studies have investigated whether genetic variations in genes involved in radiation-responsive pathways, such as ROS production, are associated with the development of adverse effects after radiation therapy, but the results have been inconsistent. Both chemotherapy and radiation therapy (RT) exert their antineoplastic effects either directly by attacking cellular macromolecules, including DNA, or indirectly by generating ROS and their by-products. Therefore, the genes selected in the present study are involved in the regulation of redox levels in cells, in cell signaling affected by the level of ROS or in the repair of DNA damage caused by ROS, or direct irradiation (6). Specifically, 1030 SNPs in 203 genes were studied for association with the level of radiation-induced AEs in BC survivors, focusing on the development of subcutaneous fibrosis. Furthermore, the association between these SNPs and the expression level of 18 genes previously found predictive of fibrosis was studied. The aim was to identify genetic markers of radiation therapy-induced adverse effects and to investigate the possible link between the genetic variation and expression profiles associated with radiosensitivity.

Methods and Materials

Ethical considerations

The Regional Committee for Medical and Research Ethics and the Norwegian Data Inspectorate approved the study. All participants gave written informed consent.

Patient samples and datasets

BC-I

Two hundred sixty-seven women were treated with either a fractionation pattern of 4.3 Gy*10 (n = 155) or 2.5 Gy*20 (n = 88) at the institute Norwegian Radium Hospital from 1975 to 1986. The material has previously been thoroughly described (4). The women were examined for radiation-induced AEs after a median follow-up time of 15.8 years (range, 10.7-21.8, 4.3-Gy group) and 10.4 (range, 7-22.8, 2.5-Gy group) and had blood samples drawn. Evaluated AEs included subcutaneous atrophy, telangiectasias, subcutaneous fibrosis, pleural thickening, development of costal fractures, and lung fibrosis in the irradiated areas. AEs were scored into groups according to the late effects in normal tissues—subjective, objective, management and analytic (LENT-SOMA) scoring system. The maximum score of AE was included in the analysis, and women receiving multiple treatments to the area investigated were excluded. Ninety-two women from BC-I were included in the initial analyses (median follow-up time for this subset of samples was 17.1 years), and the remaining samples were included in the validation step. All 92 women received the hypofractionated treatment of 4.3 Gy*10.

BC-II

Two hundred eighty-three women were treated for BC stage II/III with postoperative locoregional radiation therapy (2 Gy*25) at the institute Norwegian Radium Hospital from 1998 to 2002. The patient cohort has been previously described (7). The level of subcutaneous fibrosis was assessed 3 to 9 years after BC diagnosis, and blood samples were drawn. Whole-genome mRNA expression profiles from blood were available (8).

Evaluation of subcutaneous fibrosis (BC-I and BC-II) was performed by an experienced physiotherapist or oncologist and scored as none (level 1), barely palpable increased density (level 2), increased intensity and firmness (level 3), and very marked density, retraction, and fixation (level 4), based on the LENT-SOMA scoring system, and partly using an ad hoc-defined scoring system developed by the health professionals involved.

Cultured fibroblasts were available from 33 BC patients included in the so-called Aarhus postmastectomy study cohort (9). Normal fibroblasts were derived from skin biopsy specimens taken from an unirradiated area of the arm. A score as radiosensitive/radioresistant based on the development of fibrosis in the irradiated area was available for 14/33 of the BC patients. Gene expression data from irradiated fibroblasts were available as previously described (10).

DNA isolation

Leukocyte DNA was isolated from thawed blood containing ethylenediaminetetraacetate by the use of chloroform/phenol

extraction followed by ethanol precipitation using the Applied Biosystems 340A Nucleic Acid Extractor (BC-I) or the Autopure LS DNA Purification System (Gentra Systems, Inc, Minneapolis, MN) (BC-II).

Fibroblast cell lines

DNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA).

SNP genotyping

The SNPs for a subset of 92 samples from BC-I (all receiving 4.3 Gy*10) and the fibroblast cell lines were genotyped with the GenomeLab SNPstream genotyping system. In short, amplified polymerase chain reaction (PCR) products were used as a template in a second, modified, single-primer minisequencing reaction, followed by single-base extension to discriminate the variant base. The SNP data for BC-I were filtered according to the following criteria: failure rate >25% (n = 323), monomorphic and low frequent SNPs with <3 women in 1 genotype group (n = 137), and SNPs not in Hardy-Weinberg equilibrium (n = 42). Similar criteria were used to filter SNP data for the fibroblast cell lines, excluding an additional 16 monomorphic SNPs. The number of SNPs available for analysis after filtration was 528 and 512 for the BC-I and fibroblast cell lines, respectively. A more thorough description of the genotyping can be found in (6).

Seven SNPs were genotyped in BC-II, and the remainder of BC-I (n = 63 from the 4.3-Gy group and n = 88 from the 2.5-Gy group) using the TaqMan 7900HT Fast Real-Time PCR Platform, according to standard protocol, using 12.2 ng DNA per well. For rs1139793, the annealing temperature was lowered from 60°C to 58°C. Genotypes of rs2227306 were only successfully generated for a subset of the samples, and this SNP was excluded from further analysis. Information on the custom-made SNP assays is given in Table E1 (available at www.redjournal.org).

Statistical analyses

Data analyses were performed in SPSS (version 18; IBM, Armonk, NY), J-Express (http://www.molmine.com/), and R (versions 2.10 and 2.13, http://www.r-project.org).

Correlations between categorical variables were primarily evaluated by Pearson's correlation in SPSS; for 2 × 2 tables, Yates continuity correction was performed.

Mutual information score analysis and logistic regression (SNPassoc package in R), was used to assess SNP adverse effect associations, using all inheritance models (codominant, dominant, recessive, overdominant, and log-additive). Logistic regression analyses were used for the multivariate analyses, including treatment-related factors in the model. No correction for multiple testing was performed in the discovery analyses in the first 92 samples. In the validation analyses Bonferroni corrections were used.

Linear regression analyses were performed, regressing each SNP against the mRNA expression level to (*1*) analyze the effect of rs1139793 on mRNA level of *TXNRD2* both unadjusted and

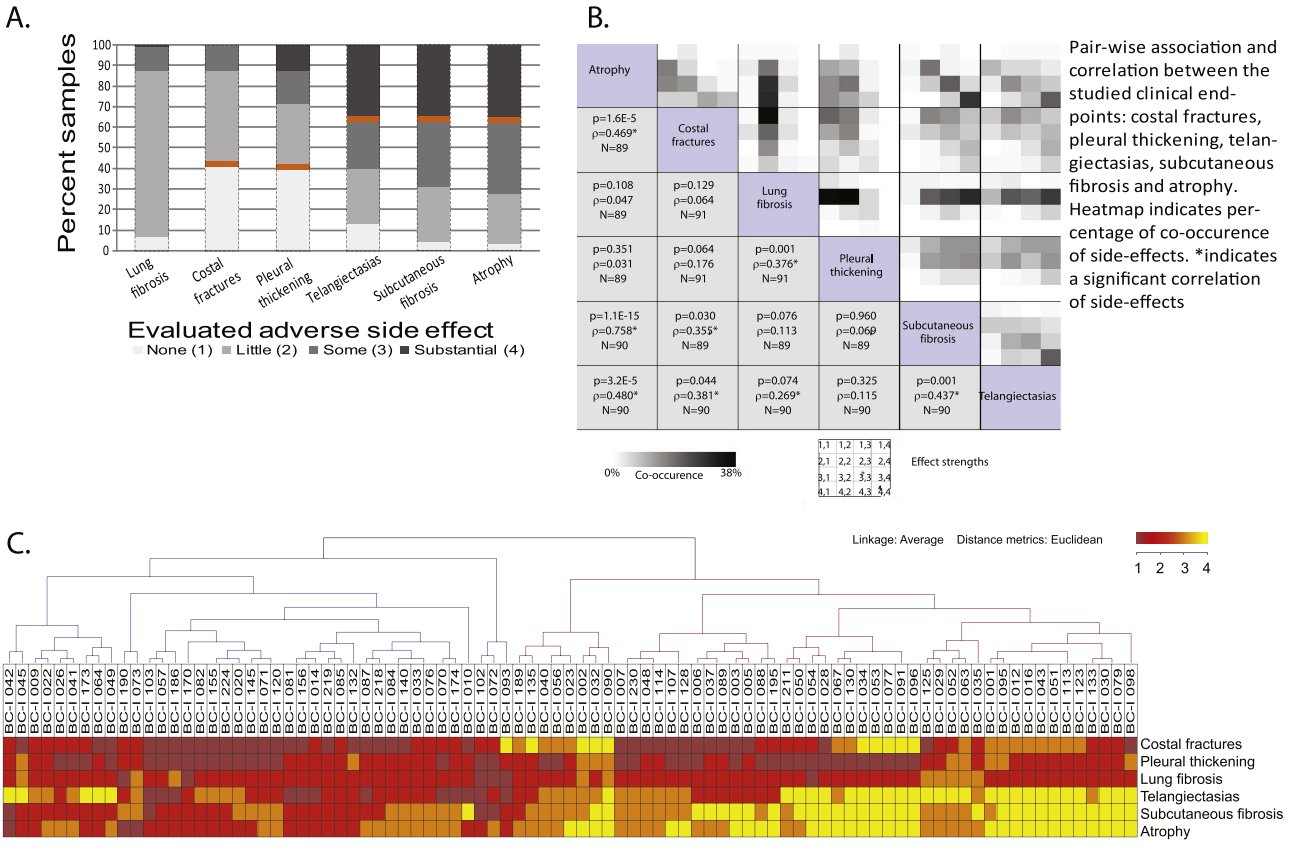

Fig. 1. (A) Distribution of the adverse effects scored as none (1), little (2), some (3), and substantial (4) for the BC-I women. (Orange lines indicate the groups used in the statistical analysis to identify genetic variation associated with level of adverse effect: below group 1 and above group 2 as indicated in Table 1.) (B) Pairwise correlation rate between the scored adverse effects. (C) Supervised clustering based on level of adverse effect using J-Express. (Scale indicates level of adverse effect.)

Table 1 Single-nucleotide polymorphisms identified as associated with level of treatment-induced subcutaneous fibrosis for BC-I

Reference sequence id (rs number)	Gene	Genotype distribution*				Mutual information score analysis
		Allele	Nr.tot	Group1*	Group2*	*P* value
744751	*TGFBR2*	CC	38	29	9	.0017
		CT	41	18	22	
		TT	12	11	1	
731465	*TGFBR2*	CC	37	28	9	.0018
		CG	40	17	22	
		GG	11	10	1	
1139793§	*TXNRD2*	CC	40	29	11	.0215
		TC	38	18	20	
		TT	3	3	0	
945222	*MGMT*	GG	46	36	10	.0282
		GA	35	17	18	
		AA	4	3	1	
2227306	*IL8*	CC	19	10	9	.0421
		CT	49	38	11	
		TT	13	6	7	
1934951	*CYP2C8*	GG	60	43	17	.0421
		GA	24	11	13	
		AA	1	1	0	
4073	*IL8*	AA	22	12	10	.0440
		AT	49	37	12	
		TT	18	8	10	

Abbreviations: CI = confidence interval; NA = not analyzed; NS = not significant; OR = odds ratio.

Listed are the results of both univariate and multivariate analyses (corrected for treatment-related factors). The *P* values are not corrected for multiple testing.

* Samples divided into 2 groups as indicated in Figure 1, for subcutaneous fibrosis, telangiectasias, and atrophy. Group 1 includes samples from women experiencing none, little, and some degree of side effects. Group 2 includes those experiencing a substantial level. For costal fractures and pleural thickening, group 1 includes samples from those experiencing none of these adverse effects versus group 2 consisting of samples from those experiencing little, some, and substantial effects.

† For the codominant model, the OR and CI are given for heterozygous in the upper line and homozygous in the lower line.

‡ In the dominant model, the effect of 1 allele masks the effect of the other when both are present. In an overdominant model, the heterozygotes have a phenotype that is more explicit or better adapted than either of the homozygotes. In a codominant model, both alleles influence the phenotype in heterozygous individuals.

§ Previous id rs2073752.

including chemotherapy as a covariate (the linear regression model in R); and (*2*) investigate the influence of ROS-related SNPs on the mRNA expression level of 18 genes previously related to fibrosis (eMAP package in R). For the second analysis, the resulting *P* values were adjusted for multiple testing by use of the Benajmini Hochberg FDR correction.

Polyphen (http://genetics.bwh.harvard.edu/pph/), SIFT (http://sift.jcvi.org/), and Provean (http://provean.jcvi.org/index.php) score analyses were used to do in silico analysis of the potential effect of the single base variation T to C in rs1139793 (Ile370Thr) in *TXNRD2* on protein function. PhyloP conservation score was extracted from UCSC for the position of rs1139793. Two-way hierarchical clustering of the BC-I survivors based on the level of AEs was performed in J-Express.

Results

Distribution of AEs

The distribution of AEs among the 4 estimated levels of damage—(*1*) none; (*2*) little; (*3*) some; and (*4*) substantial—varied considerably (Fig. 1A). Although only a few women had substantial lung fibrosis and costal fractures, more than 30% experienced substantial levels of subcutaneous fibrosis, subcutaneous atrophy, and telangiectasia. The pairwise correlation between AEs varied from 0.031 (pleural thickening and subcutaneous atrophy) to 0.758 (subcutaneous fibrosis and atrophy) (Fig. 1B). Supervised clustering of all categories of experienced adverse effects (J-Express) grouped the BC survivors into 2 main groups, wherein the majority of women experiencing level 3 or 4 for any of the evaluated adverse effects were grouped together in 1 cluster (Fig. 1C).

Impact of genetic variation in the ROS pathway on level of AEs

A total of 528 SNPs genotyped in 92 individuals from the BC-I cohort, all receiving the hypofractionated treatment, were included in the analyses. The BC-I survivors were divided in 2 groups to obtain approximately equal group sizes: either level 1 versus levels 2, 3, and 4 (for costal fractures and pleural thickening) or levels 1, 2, and 3 versus level 4 (for subcutaneous fibrosis, subcutaneous atrophy, and telangiectasias) (Fig. 1A). No analysis

Table 1 (*continued*)

Reference sequence id (rs number)	Univariated logistic regression			Multivariate logistic regression		
	P value	OR (95% CI)[†]	Genetic model[‡]	*P* value	OR (95% CI)[†]	Genetic model[‡]
744751	.0005	4.89 (1.93-12.41)	Overdominant	.0002	7.07 (2.27-21.98)	Overdominant
731465	.0006	4.92 (1.92-12.6)	Overdominant	.0004	6.84 (2.2-21.29)	Overdominant
1139793[§]	.0120	3.23 (1.27-8.24)	Overdominant	.0205	2.38 (0.82-6.84) NA	Codominant
945222	.0049	3.75 (1.46-9.63)	Overdominant	.0245	3.33 (1.14-9.71)	Dominant
2227306	.0104	0.29 (0.11-0.76)	Overdominant	.0369	0.32 (0.11-0.95)	Overdominant
1934951	.0242	3.06 (1.15-8.14)	Overdominant	.0444	3.24 (1-10.43)	Overdominant
4073	.0124	0.32 (0.13-0.8)	Overdominant	.0134	0.28 (0.1-0.79)	Overdominant

could be performed for lung fibrosis because more than 80% of BC-I women were scored at level 2.

In the univariate analysis, 1 or more SNPs were found to be associated with the level of all adverse effects by both mutual information score analysis and logistic regression modeling (not adjusted for multiple testing): costal fractures, 4 SNPs in *GCLC*, *MAPK1*, *KCNMB1,* and *COX10*; pleural thickening, 3 SNPs in *IL1β, PRKD2,* and *NR1I2*; subcutaneous fibrosis, 7 SNPs in *TGFBR2*, *TXNRD2*, *MGMT*, *CYP2C8,* and *IL8*; telangiectasia, 1 SNP in *CAT*; and subcutaneous atrophy, 3 SNPs in *IGF1R* and *IL8* (Table 1 and Table E2 [available at www.redjournal.org] for results related to fibrosis and the remaining adverse effects, respectively). The level of adverse effects was found to be associated with 1 or more treatment-related factors such as type of energy (cobalt or the combination of cobalt with electrons), surgery (mastectomy/Halsted), treatment period (before/after 1983), and time since treatment (<15 years/15-20 years/>20 years) for all clinical endpoints except pleural thickening (Table E3A, available at www.redjournal.org). Also, the treatment-related factors showed variable degrees of correlation (Table E3B, available at www.redjournal.org). Multivariate analyses, including the significant treatment-related factors for each adverse effect (Table E3C, available at www.redjournal.org) showed that the identified associations between SNPs and adverse effects were still significant, with the exception of 2 SNPs associated with the level of costal fractures (Table 1 and Table E2 [available at www.redjournal.org]).

Validation of findings related to subcutaneous fibrosis

Additional samples with data on the development of subcutaneous fibrosis were available, and the 7 SNPs in *TGFBR2*, *TXNRD2*, *MGMT*, *CYP2C8,* and *IL8* found associated with subcutaneous fibrosis in BC-I were analyzed in an independent cohort of 283 BC survivors (BC-II) and in samples from 62 patients receiving 4.3 Gy*10 and 88 patients receiving 2.5 Gy*20 (remainder of BC-I) not included in the original genotyping analyses. The SNP in *IL8* was only successfully genotyped in subsets of the samples even after multiple attempts and was excluded from further analysis. The results confirmed a strong association between rs1139793 (Ile340Thr, T<C) in *TXNRD2* and the development of fibrosis in BC-II. A significantly higher level of the T-allele was found in BC survivors with more severe grades of fibrosis in both the axilla/supraclavicular and breast/breast wall region (Fig. 2, Bonferroni

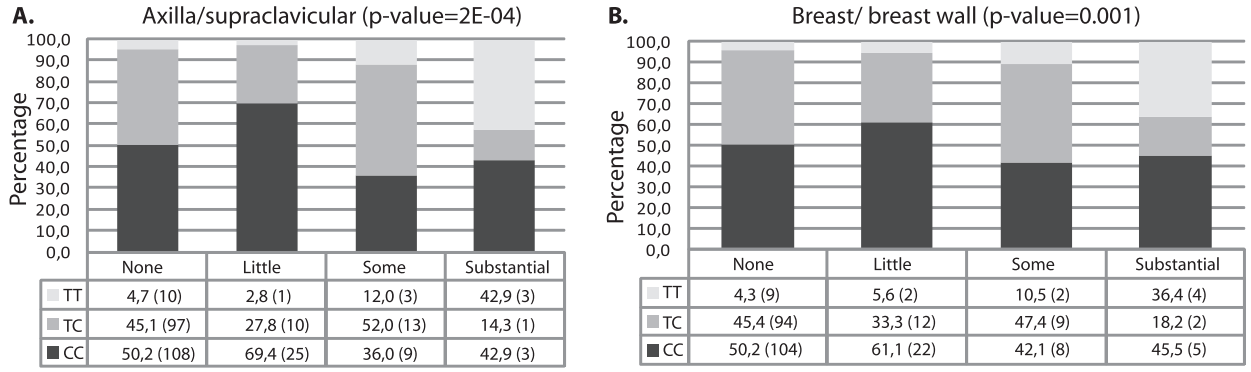

Fig. 2. Genotype distribution of rs1139793 in the *TXNRD2* gene across the different levels of subcutaneous damage fibrosis for both anatomic regions evaluated in the validation set BC-II: (A) axilla/supraclavicular. (B) breast/breast wall.

adjusted *P* values .0028 and .014, respectively). Type of chemotherapy (FEC/CMF/both/nonstandard treatment) was found significantly associated with grade of fibrosis (*P* values .00025 and .00061 for axilla/supraclavicular region and breast/breast wall, respectively). Including type of chemotherapy as a covariate, the association between rs1139793 and grade of fibrosis remained significant in a logistic regression analysis (overall $P = .007$, for the homozygous TT: *P* value .002, OR 7.53, 95% CI 2.08-27.2). Furthermore, the addition of 62 samples from patients receiving the same treatment of 4.3 Gy*10 to the initial set of 92 patients samples resulted again in a significant association between rs1139793 in *TXNRD2* and level of treatment-induced subcutaneous fibrosis (*P* value .032) with a higher level of subcutaneous fibrosis with increasing number of T-alleles. The same trend was seen for the set of 88 samples collected from patients receiving 2.5 Gy*20.

Association of rs1139793 in *TXNRD2* to expression in blood

The SNP rs1139793 in *TXNRD2* was significantly associated with the *TXNRD2* mRNA expression level in blood (in BC-II), with BC survivors homozygous for the T-allele showing the highest level of mRNA expression ($P = .021$, linear regression analysis) (Fig. 3). Previous analysis of the BC-II gene expression data had shown an association between overall gene expression level and type of chemotherapy (8). After adjustment for type of chemotherapy in a linear regression analysis, the association between rs1139793 and mRNA expression level of *TXNRD2* was further strengthened ($P = .0018$). The rs1139793 SNP is a nonsynonymous variant altering the amino acid 370 from isoleucine to threonine. In silico should be in italics since latin phrase analysis using Polyphen, Provean, and SIFT all predicted this change to be benign to protein function (Polyphen score 0.0, effect BENIGN // average Provean score -1,352, effect NEUTRAL // average SIFT score 0.243, effect TOLERATED, PhyloP score in mammals for genomic position chr22:19,868,218 1.43 indicating conservation).

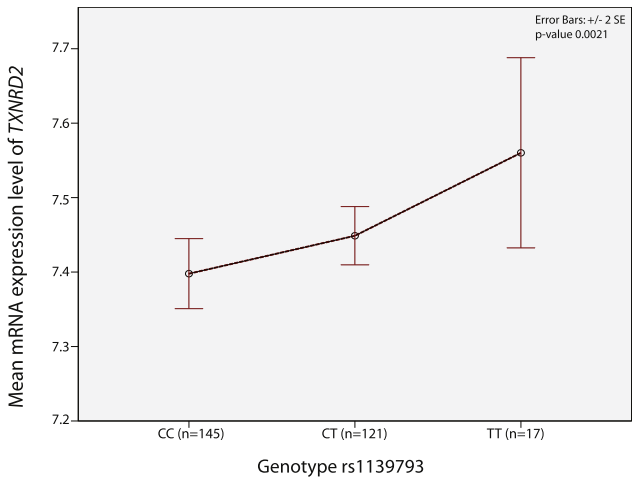

Fig. 3. Mean mRNA expression level of *TXNRD2* in blood according to genotype group for rs1139793.

Fibroblast cell lines derived from radiosensitive BC patients

A score as radioresistant/radiosensitive based on the development of fibrosis in the region irradiated during breast cancer treatment was available for 14/33 of the patients from whom we had fibroblast cell lines (4 radioresistant, 10 radiosensitive). Although too few to reach statistical significance, the reference T-allele of rs1139793 in *TXNRD2* was only found in the cell lines from the 10 radiosensitive patients (3/10 heterozygote -CT). eQTL analyses were performed in the fibroblast cell lines to investigate the influence of ROS-related SNPs on the mRNA expression level of 18 genes previously related to fibrosis by Rodningen et al (10). A total of 54 associations were identified in trans, representing 24 unique "SNP genes" related to the mRNA level of 14 of the 18 genes (Table 2, unadjusted *P* value cutoff $\leq .005$), including rs1031804 in CENPE associated with mRNA expression level of *CCND2,* and rs712831 in *EGFR*, rs1048945 in *APEX1,* and rs1871042 in GSTP1, all associated with the expression level of *ARID5B.*

Discussion

Genotype profiling of patients before cancer therapy may help to identify individuals who will not respond to a given treatment or who are likely to experience severe adverse effects. To enable

Table 2 eQTL associations identified between genetic variation in 24 reactive oxygen species-related genes and mRNA level of 14 genes linked to risk of fibrosis by Rodningen et al (10), using fibroblast cell lines from breast cancer patients (P value $\leq$.005)

SNPinfo					
rs number	MAF	Function	Gene	Expression gene	Raw P values
rs312185	0.417	Intron	AP2S1	MXRA5	.00472
rs1048945	0.061	near_gene_3__missense	APEX1	ARID5B	.00378
rs210132	0.345	unknown	BAK1	MT1H	.000715
				MT1F	.00108
				MT1X	.00251
rs1031804	0.463	Intron	CENPE	CCND2	.000336
rs718768	0.267	unknown	EGF	MT1H	.00123
				MT1X	.0013
				ARID5B	.0022
				MT1F	.00288
rs712831	0.212	Intron	EGFR	ARID5B	8.40E-05
				LUM	.00037
				MT1X	.00148
				MXRA5	.00215
rs909351	0.034	Intron	GSR	NF1	.00133
rs1695	0.409	missense	GSTP1	NF1	.0045
rs1871042	0.433	Intron		ARID5B	.00325
rs749174	0.409	Intron		NF1	.0045
rs2289807	0.031	Intron	MAPK8	NF1	.00244
rs1035413	0.121	Intron	MAPK9	ARID5B	.00338
rs1042371	0.031	untranslated_3	MYB	LUM	.00191
				NF1	.0027
				MT1X	.0039
rs967835	0.182	unknown		CCND2	.00328
rs2369088	0.371	Intron	NDUFA10	CXCL12	.00291
rs2266916	0.370	Intron	NOX1	TMEM47	.00159
				CXCL12	.00234
				MGC33894	.0027
				TMEM47	.00333
rs1847137	0.032	intron	NOX4	NF1	.0028
				LUM	.00311
rs317187	0.414	intron		C1S	.00476
rs1135534	0.034	near_gene_5	PDGFRA	MT1X	4.71E-05
				LUM	.000148
				C1S	.000188
				ARID5B	.000569
				MT1F	.000824
				CDON	.000947
				MT1H	.00154
rs854524	0.439	intron	PPP1R9A	CCND2	.00166
				CDON	.00287
rs1355984	0.136	intron	PRKCA	CDON	.00322
rs956952	0.125	intron		MGC33894	.00287
				CDON	.00344
rs753279	0.406	intron	PRO1580	MT1H	.00338
rs5277	0.182	coding_synon	PTGS2	ARID5B	.00491
rs1921308	0.078	intron	TANK	NF1	.0037
rs889914	0.078	intron		NF1	.0037
rs947712	0.328	intron	TGFB2	CDON	.000922
rs2842951	0.197	intron	TPMT	SLC1A3	.000786
rs1366811	0.242	intron		LUM	.00307
rs1366817	0.333	intron		CCND2	.00315
rs1429374	0.288	intron	XDH	LUM	.00144
rs301289	0.179	intron	XRCC4	TMEM47	.0037

such treatment predictions, a more thorough knowledge of the influence of the patient's genetic background on both treatment response and level of AEs is needed, along with an extended understanding of the influence of genetic variation on the gene expression in both tumor cells and normal cells. The present study used 2 independent BC survivor cohorts and fibroblast cell lines from a third cohort of BC patients to analyze the influence of genetic variation in candidate genes from ROS-related pathways on the level of AEs and on gene expression of genes previously linked to fibrosis.

The latency for development of 90% of the ultimate severity and frequency of AEs has previously been estimated to be 3.2 years for fibrosis and 4.7 years for telangiectasias (11), and the latency time for heart toxicity is reported to be 10 to 15 years (3). A strength of the present study is the long median follow-up time of more than 17 years, suggesting that the maximum grade of AEs had been reached for the BC-I survivors. The tissues typically receiving higher radiation doses (eg, skin and subcutaneous tissues) had more severe levels of AEs (telangiectasies, subcutaneous atrophy, and subcutaneous fibrosis) than did the tissues receiving lower radiation doses. These results are in line with previous findings showing a dose dependency for grade of AEs after radiation therapy (12). Few studies have analyzed the relationship between different types of late effects, but a previous study found no significant correlation between level of telangiectasias and fibrosis (13). By contrast, we see a significant correlation not only between telangiectasias and fibrosis but also between several of the other studied endpoints. Also, the clustering analysis revealed 2 distinct clusters with a clear overall difference in grade of AEs, suggesting that individuals with high levels of 1 AE may also experience high levels of other AEs.

Subcutaneous fibrosis has been the focal point of genetic association studies on radiation-induced AEs in BC survivors. Genetic variation in several genes has been investigated; however, no consistent associations have been reported. In the current study, subcutaneous fibrosis was associated with the SNP rs1139793 in the *TXNRD2* gene in the BC-I cohort, and the association was confirmed in the BC-II cohort, remaining significant after adjustment for multiple testing. The reference allele (T) was more prevalent among women experiencing more severe subcutaneous fibrosis. Furthermore, linear regression showed that rs1139793 was significantly associated with mRNA expression level of *TXNRD2* in blood, and individuals homozygous for the reference allele had the highest expression levels. Although the expression level was measured in blood in our study, a previous report demonstrated high correlations between the expression of *TXNRD2* in blood and breast tissue (14). rs1139793 is located toward the C-terminal end of the protein, and how genetic variation in this position influences expression remains to be elucidated, but one may hypothesize that this SNP may be in LD with other regulatory loci not studied here. *TXNRD2* encodes the selenoprotein thioredoxin reductase 2, a key player in the defense against oxidative damage, by reducing the oxireductase enzyme thioredoxin involved in ROS scavenging (15). Knockout studies of *TXNRD2* in mice resulted in embryonic lethality, preferentially caused by deficient hematopoiesis, impairment in heart development, and increased apoptosis in the liver (16). Furthermore, knockdown of the gene has been shown to significantly increase the production of hydrogen peroxide, an ROS can be removed, altogether confirming the essential role of *TXNRD2* (15). The TXNRD2 protein resides in the mitochondria and is reported to reduce cytochrome c, a complex involved in the respiratory chain (17). To our knowledge, this is the first report of an association between genetic variation in *TXNRD2* and the development of subcutaneous fibrosis.

eQTL analyses were performed to analyze the influence of SNPs in the ROS-related genes on the mRNA level of 18 genes previously linked to fibrosis (10), by use of expression profiles from irradiated fibroblast cell lines from BC patients. We identified associations between SNPs in 24 ROS-related genes and mRNA expression of 14 of the 18 genes. Among the "SNP genes" showing associations with the mRNA expression were *APEX1* and *GSTP1*. Several studies have previously investigated genetic variations in *APEX1* and *GSTP1* in relation to subcutaneous fibrosis, all reporting nonsignificant results (4, 18-20).

In conclusion, we identified an association between subcutaneous fibrosis and the SNP rs1139793 in *TXNRD2* in a cohort of 92 BC survivors, and we confirmed the association in an independent cohort of 283 BC survivors. Furthermore, the SNP was found to be associated with the mRNA expression of *TXNRD2* in blood. eQTL analyses revealed associations between genetic variation in several ROS-related genes and mRNA expression of genes previously linked to prediction of risk of subcutaneous fibrosis.

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