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Individual Genetic Variation Might Predict Acute Skin Reactions in Women Undergoing Adjuvant Breast Cancer Radiotherapy

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Individual Genetic Variation Might Predict Acute Skin Reactions in Women Undergoing Adjuvant Breast Cancer Radiotherapy

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Abstract. Adverse skin reactions during radiotherapy (RT) are common. The aim of this study was to explore whether genetic variation might be linked to acute radiation skin reactions (ARSR). **Materials and Methods:** One hundred and nineteen women undergoing adjuvant RT for breast cancer were included. The symptoms of itching, burning and irritation were self-reported twice using the visual analogue scale. Assessments used the Radiation Therapy Oncology Group scoring system for acute RT skin reaction (RTOG scale). Blood-based single nucleotide polymorphism (SNP) analysis was performed. Thirty SNPs of well-defined functional genes were investigated. **Results:** All women were assessed with ARSR. After RT, the women self-reported itching (n=97), burning (n=64) and irritation (n=96). Two SNPs in X-Ray Repair Cross Complementing 2 gene (XRCC2) rs2040639 and interferon gamma (IFNG) rs2069705 genes were found to be associated with ARSR. **Conclusion:** An association between two SNPs and ARSR was found. The possibility of using these SNPs as prognostic biomarkers for ARSR as tools to improve the care of patients needs further investigation.

Acute radiation skin reactions (ARSRs) are common in patients treated with radiotherapy (RT) (1). While adjuvant

RT for women with breast cancer (BC) is often well tolerated, ARSRs affect many of the patients in varying degrees. Most patients experience mild ARSR, with different degrees of erythema (2). A smaller proportion (20%) experience more severe ARSR, with dry or moist desquamation which is often associated with discomfort, itching, pain and disturbed sleep patterns (3, 4). ARSR normally appears 1 to 2 weeks into the RT, accelerating during the treatment period and reaching a peak approximately 10 days after the completion of RT (5, 6).

Known risk factors for ARSR are treatment-related (such as RT dose, overall treatment time, irradiated volume, use of boost or bolus) (2) and patient-related (such as high body mass index and smoking) (7).

RT induces DNA damage leading to cell-cycle arrest and cell death (8). This can be particularly toxic to normal tissues since the timing of damage appearance can vary widely between different normal tissues and tumour (9). This in turn affects the skin, resulting in various reactions from redness to flaking of the skin and eruptions. The individual variations in ARSR suggest that genetic differences might underlie the different responses (8, 10).

Single nucleotide polymorphism (SNP) is the most common variation in DNA and may result in altered gene expression as well as different responses to external factors such as radiation and drug metabolism (11). Blood-based SNP analysis is simple, cost-effective and rapid. It can be undertaken before any treatment, utilizing common laboratory facilities. It is important to evaluate the possible role of SNPs as a prognostic biomarker in relation to the frequency and intensity of ARSR in women with BC in order to improve information and skin care strategies (12, 13).

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Key Words: Radiotherapy, breast cancer, skin reactions, single nucleotide polymorphism.

Different skin care products are in clinical use to reduce ARSR. Studies present conflicting results regarding the effects of topical agents *e.g.* steroids (6, 14, 15) calendula cream (4, 16), and hyaluronic acid (17, 18).

The aim of this study was to explore whether the early signs of ARSR in patients with BC treated with adjuvant RT, as evaluated by patients and healthcare givers, might be linked to genetic variations.

Materials and Methods

Women who underwent adjuvant RT at the Department of Oncology, Ryhov County Hospital Jönköping, Sweden from February 2011 to May 2013 were included in this study. For patient characteristics, see Table I. As there were no previous data on the association between SNP and ARSR, it was not possible to perform any study power calculation before the study started. Instead this cohort study would serve as the foundation for power calculation in future studies.

The Regional Ethical Review Board approved the study (Dnr. 2010/331-31, December 2010). The women were informed verbally and in writing by an Oncology Research Nurse about the purpose and the procedures of the study. The participants were able to withdraw from the study at any time.

Participants. One hundred and thirty women of mainly Caucasian ethnicity, older than 18 years of age and scheduled for postoperative adjuvant RT after breast-conserving surgery were invited to participate. If they accepted, a written consent form was signed. Out of 130 consecutive women invited, five declined for personal reasons and six were not able to report their symptoms. Twenty-six of the women had previously been treated with chemotherapy and were also included in the analysis (fluorouracil, epirubicin and cyclophosphamide as first treatment followed by a taxane) (Figure 1). A complete dataset on the self-reported symptoms was recorded using data from the remaining 119 women.

Radiotherapy. The whole breast, clinical target volume (CTV) with 1 cm margin to planning target volume (PTV) was treated with two parallel opposing tangential fields using Varian Linacc 2100 CD, (Varian Medical Systems, Inc, Palo Alto, CA USA). Depending on the size of the breast, 6-MV or 6-MV combined with 15-MV photons in some fields, were chosen. The treatments were prescribed to the 95% isodose according to International Commission on Radiation Units and Measurements (19), in a 3-dimensional treatment planning system (Oncentra masterplan v 4.3; Elekta AB, Stockholm, Sweden). Areas of 90-105% were accepted. The absorbed dose was 50 Gy in 25 fractions given as one fraction per day, 5 days per week with an overall treatment time of 5 weeks. Gating was not used nor bolus. However, four patients were treated with a hypofractionated RT schedule (20, 21) 42, 56 Gy in 16 fractions, one fraction per day, 5 fractions per week.

Standard skin care protocol. At the Department, the standard skin care protocol recommends the use of topical corticosteroid (0.1% Betamethasone cream) on the irradiated skin from the first sign of ARSR. The women were recommended to use the corticosteroid cream twice daily until 3 weeks past the completion of RT. If moist desquamation occurred, the use of corticosteroid was stopped, and patients were recommended to continue skin care with a hydrophilic

Table I. *Clinical characteristics of the responding women.*

Characteristic	Value
Age, n (%)	
≤50 Years	15 (13)
>50 Years	104 (87)
BMI, kg/m ²	
Median (range)	27 (20-41)
Smoking, n (%)	
Yes	14 (12)
Tumour size, n (%)	
T0	1 (1)
T1	72 (61)
T2	24 (20)
T3	9 (8)
T4	1 (1)
Tis	11 (9)
T1mi	1 (1)
Node stage, n (%)	
N0	82 (69)
N1	30 (25)
N2	7 (6)
Stage of disease, n (%)	
0	12 (11)
I	52 (43)
IA	7 (6)
IIA	19 (16)
IIB	17 (14)
IIIA	11 (9)
IIIB	1 (1)
Hormone receptor, n (%)	
ER ⁺	100 (94)
ER ⁻	6 (6)
PgR ⁺	81 (76)
PgR ⁻	25 (24)
HER2 status, n (%)	
Positive	10 (9)
Negative	96 (91)
Type of tumour, n (%)	
Ductal	80 (67)
Lobular	16 (14)
DCIS	12 (10)
Other	11 (9)
Total, n (%)	119 (100)

BMI: Body mass index; DCIS: ductal carcinoma *in situ*; ER: oestrogen receptor; HER2: human epidermal growth factor receptor 2; PgR: progesterone receptor.

body lotion or cream. In addition, from the onset of RT a basic perfume-free moisturizing cream (Essex[®] MSD, Kenilworth, NJ, USA) was recommended to be used several times daily.

Assessment of the irradiated skin. Validated instruments for the evaluation of the irradiated skin were used. The original version of the Radiation Therapy Oncology Group (RTOG) scoring system for acute RT dermatitis was used to assess the irradiated skin (22). The research nurse assessed the ARSR twice during RT: 1 week after the start, as well as in the final week of RT. Itching, burning and irritation was assessed by the patient at the same time points, using a Visual Analog Scale (VAS) (scale of 0-10) (23).

Figure 1. Flow chart of the study inclusion.

Blood samples. Venous blood (30 ml) was drawn from each patient before the start of RT. High-molecular-weight DNA was extracted from the blood using MagNa Pure LC2.0 (Roche Diagnostic, F Hoffmann-La Roche, Rotkreuz, Switzerland). The quality and quantity of DNA were determined by Nanodrop ND-1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and confirmed by Pico green method using Quant-iT™ Pico Green™ dsDNA Assay Kit (ThermoFisher Scientific). The Pico Green fluorescence intensity was determined by Hidex Sense Microplate reader (Hidex Oy, Turku, Finland). DNA (250 ng) from each patient was used as the template for SNP analysis. The identification of the SNPs was performed by Illumina Golden Gate Genotyping assay at the SNP&SEQ Technology Platform, Uppsala University, Sweden (<http://www.genotyping.se>).

Selection and analyses of SNPs. The candidate genes and their SNPs (Table II) were selected based on well-defined functions concerning DNA damage, DNA repair, cell cycle regulation, cell death and inflammation, the hypothesis being that individual difference in ARSR might in part depend on differences in these genes. Based on this, 30 related SNPs in 28 genes were studied.

Statistical analysis. Descriptive statistics, numbers, medians and percentages, were utilized for the background variables. The genotypic and allelic frequencies were quality checked. For SNPs, all included genotypes fulfilled the Hardy–Weinberg equilibrium (HWE, chi-squared test, $p < 0.05$) as well as a minor allelic frequency $< 5\%$. Odds ratios and 95% confidence intervals were calculated for the influence of SNPs (exposure, *e.g.* A/A vs. G/G) on self-reported burning, itching and irritation (outcome *e.g.* irritation/no irritation).

This study originated as a pilot study and thus no study power calculation was made, since the number of patients was allocated from available resources at hand, before the start of the study. The smallest detectable effect with a patient population of 119 women was calculated afterwards. We found that an odds ratio ≥ 2.0 when comparing the influence of SNPs on the self-reported outcomes would be statistically significant, with a p -value of at least 0.05 and estimated power of 90% would be statistically satisfactory. Since there were 30 SNPs from 28 candidate genes analysed, there was a risk of false-positive test results, therefore the Hochberg method (24) was used to correct for multiple testing. In Table II, the p -values presented are after correction for multiple testing.

In order to evaluate which SNPs to use in the final risk analysis, the set of alleles (*e.g.* A/A vs. G/G) were associated with the distribution of self-reported inconvenience (*e.g.* irritation/no

irritation) from treatment. Those SNPs where no associations were found (Fisher's exact test) in relation to outcome were omitted from the final analysis (odds ratios, univariable estimation with 95% confidence intervals).

To measure the self-reported experience of burning, itching and irritation, a VAS was used, where 0 was no inconvenience and 10 was the worst possible. The results of the VAS score was dichotomized to no burning/burning (VAS=0 or VAS>0), no itching/itching (VAS=0 or VAS>0) and no irritation/irritation (VAS=0 or VAS>0). The rationale for this dichotomization was that most of the patients reported low values on the scales.

The RTOG scores (Figure 2) were dichotomized in the analysis to RTOG 0/1 (no or mild ARSR) versus RTOG 2 to 4 (severe ARSR). The statistical software for genetic analysis was SAS® Genomics for Windows, ver.9.4 and JMP® Genomics for Windows, ver. 7.0. (SAS Institute Inc., Cary, NC, USA).

Results

Assessment of ARSR and related symptoms. It was possible to evaluate the acute symptoms of itching, burning and irritation as well as RTOG scoring for all 119 of the patients.

None of the patients were assessed as having any grade of ARSR nor did they report any related symptoms during the first RT week (data not shown). During the final week of RT, all patients were assessed as having ARSR, mostly scored as RTOG 1 or 2. The median score for burning and irritation was 2, whereas it was higher for irritation, with a median of 5. At this time, many of the patients ($n=97$, 82%) reported itching (VAS scores ranging from 0 to 10), while 96 (81%) reported irritation. About half of the patients ($n=64$, 53%) reported a burning sensation from the irradiated skin (Figure 2).

Fourteen women (12%) were current smokers and 26 (23%) had previously been treated with chemotherapy. Neither smoking status nor chemotherapy before RT had an influence on acute toxicity of the skin. No association was found between high body mass index and severe ARSR between VAS scores for related symptoms or RTOG score (data not shown).

SNPs and ARSR. Out of the 114 women for whom SNP analysis was possible, 77 (68%) were prescribed topical

Table II. Single nucleotide polymorphisms (SNPs) of genes analyzed in this study

Gene	SNP	Encoded protein
<i>IFNG</i>	rs2069705	Interferon gamma
<i>EGFR</i>	rs2293347	Epidermal growth factor receptor
<i>ESR1</i>	rs2234693	Estrogen receptor
<i>TNFA</i>	rs1800629	Tumour necrosis factor -alfa
<i>TNFA</i>	rs1800610	Tumour necrosis factor -alfa
<i>ABCA1</i>	rs2230806	ATP-binding cassette transporter A1
<i>CCL5</i>	rs2107538	Chemokine (C-C motif) ligand 5
<i>XRCC2</i>	rs2040639	X-Ray repair cross-complementing protein 2
<i>FGFR4</i>	rs2011077	Fibroblast growth factor receptor 4
<i>LIG4</i>	rs1805386	DNA ligase 4
<i>MTHFR</i>	rs1801133	Methylenetetrahydrofolate reductase
<i>CRP</i>	rs1800947	C-reactive protein
<i>BRCA1</i>	rs1799966	Breast cancer 1
<i>RAD52</i>	rs11571424	DNA repair protein RAD 52 homolog
<i>CASP9</i>	rs1052576	Caspase 9, apoptosis-related cysteine peptidase
<i>CCL2</i>	rs2530797	Chemokine (C-C motif) ligand 2
<i>XRCC1</i>	rs25487	X-ray repair cross complementing protein 1
<i>CDKN2A</i>	rs3088440	Cyclin-dependent kinase inhibitor 2A, multiple tumour suppressor 1
<i>CCND3</i>	rs3218086	Cyclin D3
<i>GSTP1</i>	rs1695	Glutathione S-transferase gene class P1
<i>MMP2</i>	rs243865	Matrix metalloproteinase 2
<i>BRCA2</i>	rs144848	Breast cancer 2
<i>PRKDC</i>	rs1231204	DNA-activated protein kinase
<i>IL2</i>	rs6822844	Interleukin 2
<i>PRF1</i>	rs3758562	Perforin 1 (pore forming protein)
<i>IL12RB2</i>	rs3790568	Interleukin 12 receptor, beta 2
<i>ABCC5</i>	rs7636910	Multidrug resistance-associated protein 5
<i>CCL2</i>	rs1024611	Chemokine (C-C motif) ligand 2
<i>EGF</i>	rs4444903	Epidermal growth factor
<i>ATM</i>	rs1801516	Ataxia telangiectasia mutated

corticosteroid cream (0.1% Betametasone) during RT. The remaining 37 women (32%) were recommended only the moisturizing cream (Essex[®]) throughout the period of RT (Figure 3). After compensating for multiple testing, the following results remained statistically significant: One SNP, rs2069705, located in the interferon gamma (*IFNG*) gene was associated with itching. Another, rs 2040639 located in the x-ray repair cross complementing 2 gene (*XRCC2*) was found to be associated with burning (Table III).

Discussion

All women in this study experienced ARSR-related symptoms during the last week of RT for BC. We found evidence for individual genetic variations to be linked to ARSR and related symptoms. Skin reactions differed substantially between different individuals in this analysis. This has raised the hypothesis that genetic background may influence ARSR, along with other factors such as breast size, body mass index, smoking and the modality of RT (25-27).

The evidence for individual genetic variations associated with ARSR and related symptoms among this mainly Swedish patient cohort was investigated. Two SNPs out of 28 studied genes (*IFNG* and *XRCC2*) were found possibly to be associated with ARSR. The gene *XRCC2* has previously been shown to be involved in the repair of radiation-caused DNA damage (28). In this analysis, *XRCC2* rs2040639 was found to be associated with burning. One immune response gene, *IFNG*, was associated with ARSR. *IFNG* rs2069705 was found to be associated with itching. The *IFNG* rs2069750 encoded protein was found to have significant immunoregulatory functions (29). Thus, besides the statistically significant associations found (Table III), there is a biological rationale for the two SNPs found to be associated with ARSR. We have not found evidence in the literature contradicting our results for ARSR. Most published studies were concerned with late skin reactions of RT [reviewed in (30)].

The use of moisturizing creams and potent corticosteroid creams are common in RT (31). In this study, a potent corticosteroid cream was prescribed, in some cases after the attending nurse assessed the irradiated skin using the RTOG

Figure 2. Frequency of radiation-induced side-effects in 119 females during radiotherapy for breast cancer according to classification by the Radiation Therapy Oncology Group (RTOG) scoring system for acute radiotherapy-induced dermatitis. RTOG 0: No toxicity observed, no erythema, desquamation or pain. RTOG 1: Faint, dull, or bright erythema, psoriasis, dry desquamation, mild oedema. RTOG 2: Severe erythema, at least one moist desquamation of small size, moderate oedema. RTOG 3: Severe or confluent moist desquamation. RTOG 4: Ulceration, haemorrhage.

Table III. Genes and single nucleotide polymorphisms (SNPs) significantly associated with skin itching and burning as reactions to radiotherapy.

SNP	Genotype	Symptom, n (%) [*]		OR	95% CI	p-Value
		Reaction	No reaction			
<i>IFNG</i> rs 2069705	AA	7 (16%)	37 (84)	Ref		0.01
	AG	23 (44%)	29 (56)	4.2	0.9-10.6	
	GG	7 (37%)	12 (63)	3.1	1.6-11.1	
<i>XRCC2</i> rs 2040639	AA	14 (58)	10 (42)	Ref		0.007
	AG	31 (50)	31 (50)	6.7	1.9-23.7	
	GG	5 (17)	24 (83)	4.8	1.6-14.2	

CI: Confidence interval; *IFNG*: interferon gamma; OR: odds ratio; *XRCC2*: X-ray repair cross-complementing protein 2. ^{*}Itching for *IFNG*, burning for *XRCC2*.

scale. Our results show that when a corticosteroid cream was given when ARSR occurred (Figure 3), the symptoms continued and were still reported at the end of the treatment. RT mobilises host immune response cell accumulation at the radiation site (32, 33). When these cells accumulate, corticosteroid might not be able to inhibit their mediated functions. Since corticosteroid cream influences immune response, the lack of effects of corticosteroid cream after the onset of ARSR supports our assumption (34).

The strengths of the study are that the patients were included consecutively, and the women were treated with similar RT regimens. We used validated instruments, patient-reported and healthcare provider-reported data, as well as biological data, to provide information about ARSR. We report the findings according to the STrengthening the Reporting of Genetic Association studies in Radiogenomics (STROGAR) reporting when applicable (35).

Since the studied population in our case was relatively small, a larger study would more accurately determine the association between genetic background and ARSR after RT. This is emphasized by the impact of multiple testing making the significance more uncertain, since the standard errors are dependent on sample size and therefore a correction for multiple testing will sharpen the acceptable *p*-value limits, when the sample is relatively small. With a small sample size, the risk of missing important SNPs with a lower frequency is obvious. Other weaknesses of the study are that we used only two measurement points in addition to self-reported smoking status. Moreover, no assessments were performed after RT when ARSR typically peaks. The four patients who received a hypofractionated schedule presented no difference in reported outcomes due to similar radiation intensity or by chance. This could be due to the small number of patients treated with hypofractionation.

Figure 3. A: Starting day during radiotherapy of corticosteroid cream treatment of acute radiation skin reactions. Forty-two women did not receive corticosteroid cream. B: Self-reported symptoms of burning, itching and irritation, as reported on a 10-grade Visual Analog Scale (VAS) among 119 females during the last week of radiotherapy for breast cancer. The side-effects are irrespective of treatment with moisturizer or corticosteroid cream.

Even though new RT techniques, or developed devices for treatment have improved the accuracy of the RT, there are no guarantees that early symptoms such as ARSR will not be experienced during the treatment (10, 36).

However, our results support the hypothesis that the genetic background might influence individual differences in ARSR. The results should not be used for any change of RT but could rather be a future tool to individualize the intensity of care for the side-effects from the treatment. Future studies could be directed to explore the clinical relevance of blood-based SNPs as prognostic biomarkers for ARSR.

Conclusion

The use of rapid and stable biomarkers to identify patients with an increased risk of ARSR would be helpful to personalize treatment and self-care advice. We detected an association between blood-based SNPs and ARSR in patients treated with

adjuvant RT for BC. The possibility of using these specific SNPs in the clinical situation needs further investigation.

Conflicts of Interest

The Authors declare no conflicts of interest in regard to this study.

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