Brief report

FGFR2 genotype and risk of radiation-associated breast cancer in Hodgkin lymphoma

Yussanne P. Ma,[1] Flora E. van Leeuwen,[2] Rosie Cooke,[3] Annegien Broeks,[4] Victor Enciso-Mora,[1] Bianca Olver,[1] Amy Lloyd,[1] Peter Broderick,[1] Nicola S. Russell,[5] Cecile Janus,[6] Alan Ashworth,[7] Richard S. Houlston,[1] and Anthony J. Swerdlow[3]

[1]Section of Cancer Genetics, Institute of Cancer Research, Sutton, United Kingdom; [2]Department of Epidemiology, Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands; [3]Section of Epidemiology, Institute of Cancer Research, Sutton, United Kingdom; [4]Department of Experimental Therapy, Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands; [5]Department of Radiation Oncology, Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands; [6]Erasmus Medical Center, Daniel den Hoed, Rotterdam, The Netherlands; and [7]The Breakthrough Breast Cancer Research Centre, London, United Kingdom

Women treated at young ages with supradiaphragmatic radiotherapy for Hodgkin lymphoma (HL) have a highly increased risk of breast cancer. For personalized advice and follow-up regimens for patients, information is needed on how the radiotherapy-related risk is affected by other breast cancer risk factors. Genome-wide association studies have identified 14 independently replicated common single nucleotide polymorphisms that influence breast cancer risk. To examine whether these variants contribute to risk of radiation-associated breast cancer in HL, we analyzed 2 independent case-control series, from the United Kingdom and The Netherlands, totaling 693 HL patients, 232 with breast cancer and 461 without. rs1219648, which annotates the *FGFR2* gene, was associated with risk in both series (combined per-allele odds ratio = 1.59, 95% confidence interval: 1.26-2.02; $P = .000111$). These data provide evidence that genetic variation in *FGFR2* influences radiation-induced breast cancer risk. (*Blood*. 2012;119(4): 1029-1031)

Introduction

Radiotherapy and combination chemotherapy have dramatically improved the prognosis of Hodgkin lymphoma (HL), but at the price of raised risks of secondary malignancy. For women treated with supradiaphragmatic radiotherapy at young ages, breast cancer is a particular hazard[1-3]

It has long been postulated that genetically determined responses may affect the severity of late complications after radiotherapy. Certain rare inherited cancer syndromes are typified by radiosensitivity[4] and cancer patients and some of their first-degree relatives show greater in vitro radiosensitivity than healthy individuals.[5]

Genome-wide association studies (GWAS) have identified 14 independent common single nucleotide polymorphisms (SNPs) that show consistent associations with female breast cancer risk.[6-13] To examine whether these variants contribute to breast cancer risk after radiotherapy, we analyzed blood samples from 2 case-control series. The discovery phase comprised 449 women with HL treated with supradiaphragmatic radiotherapy in England and Wales during 1963-2003 at ages < 36 years: 140 had breast cancer after HL treatment (the "cases") and 309 had had no solid cancer after HL (the "controls"). The replication series was 244 female Dutch HL patients treated with supradiaphragmatic radiotherapy during 1965-1997 at ages < 41 years: 92 cases and 152 controls. Details are given in supplemental Methods and supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Methods

DNA was extracted from EDTA-venous blood samples using conventional methodologies and quantified using PicoGreen (Invitrogen). To evaluate the impact of variation at the 14 breast cancer risk loci (detailed in supplemental Methods) on breast cancer risk in HL patients, we derived genotypes at these loci for the discovery phase patients using previously generated HL GWAS data[14] using Illumina Infinium HD Human660-Quad BeadChips. Replication genotyping was performed using KasPar allele-specific PCR (KBioscience) and analyzed in an Applied Biosystem ABI7900HT system.

The relative risk of breast cancer associated with SNP genotype was estimated by calculating odds ratios (ORs). All statistical tests were 2-sided. After analyzing the United Kingdom and Dutch datasets separately, we pooled them to increase power. Meta-analysis was performed under a fixed-effects model, estimating the Cochran Q statistic to test for heterogeneity and the I^2 statistic to quantify variation between studies.

SNP genotypes were obtained for all 14 SNPs for $> 95\%$ of patients in the discovery phase (supplemental Table 2). Minor allele frequencies of the 14 SNPs in the HL patients were similar to those in 2 published population control sets (supplemental Table 2), providing no evidence for an association between genetic variation at any of the 14 loci and HL risk (ie, $P > .05$).

Results and discussion

rs1219648 genotype frequencies were significantly different between cases and controls. Specifically, in the discovery series there

Submitted October 12, 2011; accepted November 8, 2011. Prepublished online as *Blood* First Edition paper, December 5, 2011; DOI 10.1182/blood-2011-10-383380.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Table 1. Risk of radiation-associated breast cancer associated with rs1219648 in Hodgkin lymphoma patients

	Discovery				Replication				Combined	
	Breast cancer in HL patient				Breast cancer in HL patient					
Genotype	+	–	OR (95% CI)	*P*	+	–	OR (95% CI)	*P*	OR (95% CI)	*P*
AA	41	132	1.00	—	29	57	1.00		1.00	—
AG	68	146	1.50 (0.95-2.36)	.08	45	78	1.13 (0.64-2.02)	.67	1.35 (0.94-1.93)	.10
GG	31	31	3.22 (1.75-5.92)	.000167	18	17	2.08 (0.94-4.63)	.072	2.74 (1.69-4.45)	.000444
Per allele			1.73 (1.29-2.34)	.000273			1.37 (0.93-2.02)	.11	1.59 (1.26-2.02)	.000111

OR indicates odds ratio; CI, confidence interval; and —, not applicable.

was an overrepresentation of the minor, G allele, in HL patients with breast cancer (OR = 1.73; $P = 000273$, Table 1). This association was dose-dependent, with highest risks in patients homozygous for the G allele. The replication series provided additional support for this association, albeit nonsignificantly (Table 1), and in pooled data from both series there was a highly significant relation (per-allele OR = 1.59, 95% confidence interval [CI]: 1.26-2.02, $P = .000111$, $P_{het} = 0.35$, $I^2 = 0\%$; Table 1), remaining almost unchanged in analyses restricted to invasive breast cancers (ie, excluding cases of ductal carcinoma in situ: n = 28) and remaining significant after Bonferroni correction for multiple SNP testing. The OR is larger than that for breast cancer in relation to *FGFR2* genotype in the general population (1.26 per allele[12]). The effect was somewhat greater in patients first treated before age 20 (OR = 1.70 [1.16-2.50]) than those treated at older ages (OR = 1.48 [1.09-2.00]), and in patients who had not received alkylating chemotherapy or ≥ 5-Gy pelvic radiotherapy (OR = 1.79 [1.25-2.57]), than those who had received either of these (OR = 1.45 [1.00-2.11]; not in table).

rs1219648 maps to a 25-kb region of linkage disequilibrium within the second intron and containing exon 2 of the *FGFR2* gene. *FGFR2* is overexpressed in estrogen receptor–positive tumors,[15] *FGFR2* signaling having oncogenic effects.[16] The functional basis of the rs1219648 association with breast cancer appears to be through allele-specific up-regulation of *FGFR2*, thereby increasing the propensity for tumor formation.[17] While one study has shown radiosensitizing effects of differential *FGFR2* expression in a human prostate cancer cell line,[18] the effect in breast cancer awaits study.

Because of the large risks of breast cancer occurring after supradiaphragmatic radiotherapy for HL (cumulative risks of 25% or more at 25-30 years follow-up[3]), specific follow-up clinics have been established to counsel patients.[19,20] Much is known about radiotherapy-related factors that increase risks, and ovarian-toxic treatments that decrease them,[1,3] but it is unknown whether genetic factors affect these risks further. It has been suggested that radiotherapy might constitute a particular hazard for ataxia-telangiectasia carriers,[21] but studies have been small and not shown any clear association.[22,23] Family history of breast or ovarian cancer has not been found associated with risk, based on small numbers.[24,25]

Our data provide evidence that genetic variation in *FGFR2* influences breast cancer risk in HL patients treated with radiotherapy, especially those treated at young ages and those not treated with ovarian-toxic agents: the groups with the greatest treatment-related risks. This information should improve individualization of advice to HL patients on their risks, and decisions on prophylactic mastectomy and screening regimens.

Acknowledgments

The United Kingdom case-control study thanks the study participants, study staff, and clinicians who participated in the study as listed. We are indebted to the patients and physicians who participated in the Dutch data and sample collection.

This work was supported by Leukaemia & Lymphoma Research, Cancer Research UK, and the Bobby Moore Fund (C1298/A8362, R.S.H.) for genetic analyses; Breakthrough Breast Cancer and the European Union for United Kingdom sample and data acquisition; and the Dutch Cancer Society (NKI 2001-2425 and NKI 2004-3068, F.E.v.L.) for Dutch sample and data acquisition.

Authorship

Contribution: Y.P.M., R.S.H., and A.J.S. drafted the manuscript; Y.P.M. and V.E.-M. performed statistical and bioinformatic analyses; F.E.v.L. designed the Dutch National Cancer Institute (NKI) study and obtained financial support; R.C., C.J., A.A., and A.J.S. provided samples and data from a study conducted at the Institute of Cancer Research; A.B. coordinated collection and preparation of NKI samples; B.O. and A.L. performed genotyping; P.B. was responsible for sample coordination and laboratory analyses; N.S.R. and C.J. were involved in identification and inclusion of Dutch cases, study design, review board approval, and clinical implementation; R.S.H. designed the study and obtained financial support; and all authors contributed to the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Prof Anthony Swerdlow, DSc, Section of Epidemiology, Sir Richard Doll Building, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, United Kingdom; e-mail: anthony.swerdlow@icr.ac.uk.

References

1. van Leeuwen FE, Swerdlow AJ, Travis LB. Second cancers after treatment of Hodgkin lymphoma. In: Hoppe RT, Mauch PT, Armitage JO, Diehl V, Weiss LM, eds. *Hodgkin Lymphoma.* Philadelphia, PA: Wolters Kluwer, Lippincott Williams & Wilkins; 2007;347-370.
2. Hodgson DC, van Leeuwen FE. Second malignancy risk after treatment of Hodgkin lymphoma. In: Engert A, Horning SJ, eds. *Hodgkin Lymphoma.* Berlin Heidelberg, Germany: Springer-Verlag; 2011;305-331.
3. De Bruin ML, Sparidans J, van't Veer MB, et al. Breast cancer risk in female survivors of Hodgkin's lymphoma: lower risk after smaller radiation volumes. *J Clin Oncol.* 2009;27(26):4239-4246.
4. Gatti RA. The inherited basis of human radiosensitivity. *Acta Oncol.* 2001;40(6):702-711.

5. Burrill W, Barber JB, Roberts SA, Bulman B, Scott D. Heritability of chromosomal radiosensitivity in breast cancer patients: a pilot study with the lymphocyte micronucleus assay. *Int J Radiat Biol*. 2000;76(12):1617-1619.
6. Thomas G, Jacobs KB, Kraft P, et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat Genet*. 2009;41(5):579-584.
7. Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet*. 2007;39(7):865-869.
8. Ahmed S, Thomas G, Ghoussaini M, et al. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet*. 2009;41(5):585-590.
9. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*. 2007;447(7148):1087-1093.
10. Fletcher O, Johnson N, Orr N, et al. Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. *J Natl Cancer Inst*. 2011;103(5):425-435.
11. Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet*. 2008;40(6):703-706.
12. Gold B, Kirchhoff T, Stefanov S, et al. Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. *Proc Natl Acad Sci U S A*. 2008;105(11):4340-4345.
13. Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet*. 2009;41(3):324-328.
14. Enciso-Mora V, Broderick P, Ma Y, et al. A genome-wide association study of Hodgkin's lymphoma identifies new susceptibility loci at 2p16.1 (REL), 8q24.21 and 10p14 (GATA3). *Nat Genet*. 2010;42(12):1126-1130.
15. Turner N, Lambros MB, Horlings HM, et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene*. 2010;29(14):2013-2023.
16. Haugsten EM, Wiedlocha A, Olsnes S, Wesche J. Roles of fibroblast growth factor receptors in carcinogenesis. *Mol Cancer Res*. 2010;8(11):1439-1452.
17. Meyer KB, Maia AT, O'Reilly M, et al. Allele-specific up-regulation of FGFR2 increases susceptibility to breast cancer. *PLoS Biol*. 2008;6(5):e108.
18. Matsubara A, Teishima J, Mirkhat S, et al. Restoration of FGF receptor type 2 enhances radiosensitivity of hormone-refractory human prostate carcinoma PC-3 cells. *Anticancer Res*. 2008;28(4B):2141-2146.
19. Diller L, Medeiros Nancarrow C, Shaffer K, et al. Breast cancer screening in women previously treated for Hodgkin's disease: a prospective cohort study. *J Clin Oncol*. 2002;20(8):2085-2091.
20. Howell SJ, Searle C, Goode V, et al. The UK national breast cancer screening programme for survivors of Hodgkin lymphoma detects breast cancer at an early stage. *Br J Cancer*. 2009;101(4):582-588.
21. Ramsey J, Birrell G, Lavin M. Breast cancer and radiotherapy in ataxia-telangiectasia heterozygote. *Lancet*. 1996;347(9015):1627.
22. Offit K, Gilad S, Paglin S, et al. Rare variants of ATM and risk for Hodgkin's disease and radiation-associated breast cancers. *Clin Cancer Res*. 2002;8(12):3813-3819.
23. Broeks A, Russell NS, Floore AN, et al. Increased risk of breast cancer following irradiation for Hodgkin's disease is not a result of ATM germline mutations. *Int J Radiat Biol*. 2000;76(5):693-698.
24. Bhatia S, Meadows AT, Robison LL, Late Effects Study Group. Family history of patients with breast cancer after treatment of Hodgkin's disease in childhood. *Lancet*. 1997;350(9081):888-889.
25. Hill DA, Gilbert E, Dores GM, et al. Breast cancer risk following radiotherapy for Hodgkin lymphoma: modification by other risk factors. *Blood*. 2005;106(10):3358-3365.
26. Broderick P, Cunningham D, Vijayakrishnan J, et al. IRF4 polymorphism rs872071 and risk of Hodgkin lymphoma. *Br J Haematol*. 2009;148(3):413-415.