Short Communication

Association of Genetic Variants at 8q24 with Breast Cancer Risk

Olivia Fletcher,[1] Nichola Johnson,[1] Lorna Gibson,[2] Ben Coupland,[1] Agnes Fraser,[2] Angela Leonard,[2] Isabel dos Santos Silva,[2] Alan Ashworth,[1] Richard Houlston,[3] and Julian Peto[2,4]

[1]Breakthrough Breast Cancer Research Centre, Institute of Cancer Research; [2]Non-communicable Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Section of Cancer Genetics; and [4]Cancer Research UK Epidemiology and Genetics Unit, Institute of Cancer Research, Sutton, Surrey, United Kingdom

Abstract

Recent whole genome association studies of prostate, breast, and colorectal cancer have identified susceptibility loci on 8q24. We genotyped three variants associated with prostate cancer (rs10090154, rs13254738, and rs7000448), one associated with both prostate and colorectal cancer (rs6983267), and one associated with breast cancer (rs13281615) in a series of 1,499 breast cancer cases and 1,390 controls. 1,267 (85%) of the cases had two primary breast cancers. Our analysis provides further evidence of the relationship between rs13281615 and risk of breast cancer, with heterozygote odds ratio (OR) 1.30 95% confidence interval (CI) 1.09-1.54 and homozygote OR 1.52 (95% CI, 1.22-1.89; P_{trend} = 0.00003), and confirms the prediction that the risk is substantially higher in this genetically enriched series (OR per allele, 1.24; 95% CI, 1.12-1.38) than in a large series of mainly unselected cases (reported OR per allele, 1.08; 95% CI, 1.05-1.11). We observed a protective effect of rs13254738 for breast cancer (allelic OR, 0.88; 95% CI, 0.78-0.98; P = 0.02), which is supported by the Cancer Genetic Markers of Susceptibility data (pooled allelic OR, 0.88; 95% CI, 0.81-0.96; P = 0.003). None of the other three single nucleotide polymorphisms, two associated with prostate (rs10090154 and rs7000448) and one with both prostate and colorectal cancers (rs6583267), was associated with breast cancer risk in our study. This evidence of a protective effect for breast cancer of one variant (rs13254738) that has been associated previously with a 1.25-fold increased risk of prostate cancer, with no effect for the two other variants, indicates that the effects of the risk alleles clustered at 8q24 are cancer site specific. (Cancer Epidemiol Biomarkers Prev 2008;17(3):702–5)

Introduction

Recent whole genome association studies of common epithelial cancers have identified several susceptibility loci at 8q24. Variants independently associated with prostate cancer risk (1-3) mapped to three adjacent regions at 8q24: 128.54 to 128.61 Mb (region 1), 128.14 to 128.28 Mb (region 2), and 128.47 to 128.53 Mb (region 3). Easton et al. (4) reported an association between rs13281615 and breast cancer. This single nucleotide polymorphism (SNP) lies at 128.42 Mb, ~50 kb centromeric to the ''region 3'' block of linkage disequilibrium identified by the prostate cancer studies. Subsequently, two SNPs 5.86 kb apart that also mapped to prostate cancer region 3, rs6983267 and rs10505477, were identified as colorectal cancer susceptibility alleles (5-7). To evaluate the effect of these loci on breast cancer risk, we genotyped one of the SNPs from region 1 (rs10090154), one from region 2 (rs13254738), the SNP identified previously as a breast cancer susceptibility allele (rs13281615), and two SNPs from region 3 (rs6983267 and rs7000448) in a genetically enriched series of 1,499 breast cancer cases and 1,390 healthy controls.

Materials and Methods

Cases. 1,114 cases whose first breast cancer was diagnosed after 1970 and before age 66 were ascertained through the English and Scottish cancer registries (median age at first diagnosis, 48 years; range, 25-65). The majority (882) had two primary breast cancers, and a further 103 had at least one affected first-degree relative. An additional 385 bilateral breast cancer cases whose first breast cancer was diagnosed after 1967 and before age 71 were ascertained through the National Cancer Research Network (median age at first diagnosis, 50 years; range, 24-70).

Controls. 899 controls who were friends or nonblood relatives of patients with malignancies were recruited

Received 9/14/07; revised 12/20/07; accepted 12/21/07.

Grant support: Cancer Research UK and Breakthrough Breast Cancer.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: O. Fletcher and N. Johnson contributed equally to this work.

Requests for reprints: Julian Peto, Non-communicable Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom. Phone: 207-927-2455; Fax: 207-580-6897. E-mail: julian.peto@lshtm.ac.uk

doi:10.1158/1055-9965.EPI-07-2564

through cases as part of the British Breast Cancer Study and National Cancer Research Network studies. Additional controls who were healthy women participating in a randomized trial of mammographic screening (the AGE trial http://www.icr.ac.uk/research/research_sections/epidemiology/epidemiology_teams/cancer_screening_evaluation_unit/age_trial/index.shtml) were recruited through English mammography clinics (n = 491). None of the controls had a personal history of malignancy at the time of ascertainment.

All cases and controls were Caucasians resident in the United Kingdom. Written informed consent was obtained from all participants, and the study was approved by the South East Multicentre Research Ethics Committee.

Genotyping. DNA was extracted from blood samples using conventional methodologies and quantified using PicoGreen (Invitrogen). Genotyping of samples for rs13254738, rs13281615, rs6983267, and rs7000448 was done by KBioscience (http://www.kbioscience.co.uk) using their proprietary in house competitive allele-specific PCR system (KASPar), a competitive allele-specific PCR SNP genotyping system that uses FRET quencher cassette oligonucleotides. rs10090154 was genotyped using a TaqMan assay (C__26755480_10; Applied Biosystems).

Statistical Methods. Control genotypes were tested for departure from Hardy-Weinberg equilibrium. Unadjusted odds ratios (OR) with two-sided exact P values and likelihood ratio trend tests were calculated using Stata 9 (Stata). Per allele ORs were estimated by logistic regression. Data on SNPs in the 8q24 region from the Cancer Genetic Markers of Susceptibility breast cancer genome-wide association study (8) were accessed at http://cgems.cancer.gov/data/.

Results

Call rates were 94%, 97%, 97%, 97%, and 98% for rs10090154, rs13254738, rs1328615, rs7000448, and

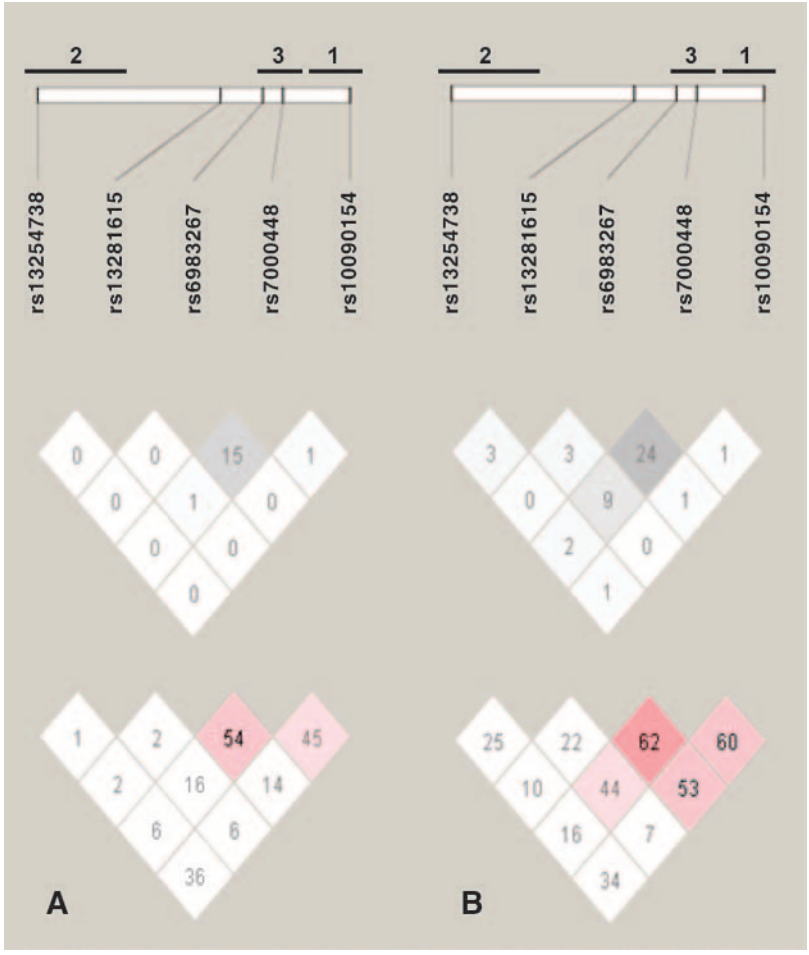

Figure 1. Schematic view of the locations and the linkage disequilibrium between SNPs tested across the 8q24 region. Regions 2, 3, and 1 cover chromosome positions, 128.14 to 128.28, 128.47 to 128.53, and 128.54 to 128.61 Mb, respectively. The linkage disequilibrium plots show estimates of the square of the correlation coefficient (r^2) in grayscale (black = 1, white = 0), and D' values in a black-to-white (higher to lower linkage disequilibrium) gradient, calculated for each pairwise comparison of SNPs derived by the Haploview (v3.32) program. Plots were generated using (**A**) this study group and (**B**) SNP genotyping data from HapMap Data Rel21/phaseII Jan07, on NCBI B35 assembly, dbSNP b125.

Table 1. Risk of breast cancer associated with 8q24 SNPs

Locus/SNP	Genotype	*n* (%) Cases	*n* (%) Controls	OR (95% CI)	P_{trend}
Prostate region 1					
rs10090154	CC	1177 (83.0)	1086 (83.0)	Reference	
	CT	225 (15.9)	212 (16.2)	0.98 (0.79-1.21)	
	TT	17 (1.2)	10 (0.8)	1.57 (0.68-3.85)	
				Per allele 1.03 (0.86-1.24)	0.74
Prostate region 2					
rs13254738	AA	708 (48.3)	601 (45.2)	Reference	
	AC	633 (43.2)	583 (43.8)	0.92 (0.79-1.08)	
	CC	124 (8.5)	146 (11.0)	0.72 (0.55-0.95)	
				Per allele 0.88 (0.78-0.98)	0.02
Breast region					
rs13281615	AA	435 (29.6)	487 (36.3)	Reference	
	AG	730 (49.7)	629 (46.9)	1.30 (1.09-1.54)	
	GG	305 (20.8)	225 (16.8)	1.52 (1.22-1.89)	
				Per allele 1.24 (1.12-1.38)	0.00003
Prostate region 3					
rs6983267	GG	408 (27.6)	371 (27.8)	Reference	
	GT	734 (49.6)	653 (48.9)	1.02 (0.85-1.22)	
	TT	338 (22.8)	312 (23.4)	0.99 (0.80-1.22)	
				Per allele 0.99 (0.90-1.10)	0.91
rs7000448	CC	596 (40.4)	559 (41.8)	Reference	
	CT	682 (46.2)	601 (44.9)	1.06 (0.90-1.25)	
	TT	199 (13.5)	178 (13.3)	1.05 (0.83-1.33)	
				Per allele 1.03 (0.93-1.15)	0.54

NOTE: Test of Hardy-Weinberg: P = 0.92, 0.80, 0.37, 0.45, and 0.41 for rs10090154, rs13254738, rs13281615, rs6983267, and rs7000448.

rs6983267. Genotype frequencies in the control groups (separately and combined) were consistent with Hardy-Weinberg equilibrium. The relative locations of the five SNPs within 8q24 and corresponding linkage disequilibrium structure between loci are shown in Fig. 1. The only correlation was weak linkage disequilibrium between the two SNPs from region 3 (rs6983267 and rs7000448) in accordance with data from HapMap. Table 1 shows genotype frequencies in cases and controls. The minor allele of the region 2 SNP (rs13254738) showed a modest protective effect (allelic OR, 0.88 [95% confidence interval (95% CI)], 0.78-0.98; P = 0.02) for breast cancer. Neither the region 1 SNP (rs10090154) nor the region 3 SNPs (rs7000448 and rs6983267) were associated with breast cancer risk. In contrast, rs13281615 showed a highly significant association with breast cancer risk (P_{trend} = 0.00003) with heterozygote and homozygote ORs of 1.30 (95% CI, 1.09-1.54) and 1.52 (95% CI, 1.22-1.89), respectively. The association remained significant (P_{trend} = 0.0001) after excluding 90 of the 1,499 cases (6.0%) that were included in stage I of the breast cancer genome-wide association study reported by Easton et al. (4).

The number of common genetic variants for which evidence of a disease association reaches genome wide significance is increasing and the issue of when and how to test for epistatic effects will become increasingly important. For whole genome association studies, Marchini et al. (9) proposed first carrying out single-locus tests and then examining pairwise combinations for all loci that pass a relatively modest level of significance. We tested for an interaction between the two loci at 8q24 for which there was a main effect in this study. Table 2 shows genotype data for both loci combined. There appears to be a modest trend, the per allele OR for the breast cancer SNP rs13281615 increasing from 1.07 (95% CI, 0.75-1.73; P = 0.70) in rare homozygotes for the prostate cancer SNP rs13254738 to 1.36 (95% CI, 1.16-1.59; P = 0.00007) in common homozygotes, although a likelihood ratio test comparing models with and without an interaction term was not significant (P = 0.13).

Table 2. Risk of breast cancer associated with rs13281615 stratified by genotype for rs13254738

rs13254738	rs13281615	*n* (%) Cases	*n* (%) Controls	OR (95% CI)	P_{trend}
AA	AA	196 (28.1)	219 (36.9)	Reference	
	AG	345 (49.5)	280 (47.1)	1.38 (1.06-1.78)	
	GG	156 (22.4)	95 (16.0)	1.83 (1.32-2.56)	
				Per allele 1.36 (1.16-1.59)	0.00007
AC	AA	196 (31.5)	217 (37.8)	Reference	
	AG	305 (49.0)	256 (44.6)	1.31 (1.01-1.71)	
	GG	122 (19.6)	101 (17.6)	1.34 (0.95-1.88)	
				Per allele 1.18 (1.01-1.38)	0.04
CC	AA	35 (28.7)	44 (30.3)	Reference	
	AG	65 (53.3)	77 (53.1)	1.06 (0.59-1.92)	
	GG	22 (18.0)	24 (17.2)	1.15 (0.52-2.54)	
				Per allele 1.07 (0.75-1.53)	0.70

Discussion

Whole genome association studies have now been reported for prostate, breast, and colorectal cancers, and risk alleles for all these three common epithelial cancers have been found within a 0.5 Mb region of 8q24 that contains no known genes. There is a high degree of site specificity of familial cancer risks, but epidemiologic studies are consistent with some association between breast and prostate cancer susceptibility (10, 11), and we and others have shown that low penetrance alleles such as variants in *CHEK2* contribute to such relationships (12). We therefore analyzed the effects of these loci in our genetically enriched breast cancer case series.

Our results suggest that at least one of the loci in this region has opposite effects on prostate and breast cancer. The variant for which carrier status for the minor allele was reported by Haiman et al. (3) to be associated with a 1.25-fold increased risk of prostate cancer (rs13254738) appears to confer a protective effect for breast cancer in our data (OR per allele, 0.88; 95% CI, 0.78-0.98; $P = 0.022$). The breast cancer OR estimate from the Cancer Genetic Markers of Susceptibility data (http://cgems.cancer.gov/data/; ref. 8) for rs1456315, which tags rs13254738 at $r^2 = 0.68$, is 0.88 (95% CI, 0.78-1.00; $P = 0.053$), giving a pooled significance level of $P = 0.003$. Two other variants associated with prostate cancer (rs10090154 and rs7000448) showed no effect on breast cancer risk in our data (Table 1), and Cancer Genetic Markers of Susceptibility (8) gave an OR per allele of 1.02 (95% CI, 0.84-1.25) for rs424384, which tags rs10090154 perfectly, and 0.91 (95% CI, 0.80-1.03) for rs12375310, which tags rs7000448 at $r^2 = 0.59$. The other study that has examined a SNP at 8q24 for which there is good evidence of an association with prostate cancer risk (rs1447295) found no evidence for an association with breast cancer (13). Further data on breast cancer risk are needed for the SNP associated with both prostate and colorectal cancer (rs6983267). This showed no effect on breast cancer risk in our data (OR per allele, 0.99; 95% CI, 0.90-1.10), but the Cancer Genetic Markers of Susceptibility (8) estimate for this SNP was significantly elevated (OR per allele, 1.17; 95% CI, 1.04-1.31).

Our analysis provides further confirmation of the relationship between rs13281615 and risk of breast cancer. Based on pooled data from 26,258 breast cancer cases and 26,894 controls the OR per allele for rs13281615 was estimated as 1.08 (95% CI, 1.05-1.11) by Easton et al. (4). The OR for cases with bilateral disease or a strong family history is likely to be approximately the square of this estimate (14), giving a predicted OR of ~1.17 (95% CI, 1.10-1.23) for the genetically enriched cases in our study. Our estimate (OR per allele, 1.24; 95% CI, 1.12-1.38; Table 1) is higher but statistically consistent ($P > 0.1$ for difference in ORs). The power of Easton et al. (4) to detect rs13281615 was only 3%, suggesting that the 8q24 region may contain several more breast cancer susceptibility loci.

We tested for an interaction between rs13281615 and rs13254738, the SNP for which our data suggest a protective effect. The interaction between the two risk alleles (minor allele of rs13281615 and major allele of rs13254738) was 1.14 (95% CI, 0.96-1.34). There may be a greater than multiplicative interaction between this pair of alleles but the effect, if any, is small. Our study based on genetically enriched cases had equivalent power to a study of ~6,000 unselected cases and 6,000 controls. Despite this, we lacked power to detect moderate epistatic effects, and pooling of available data (15, 16) is likely to be required to detect gene-gene interactions.

Acknowledgments

We thank Karen Kourellias and the Clinical Trials Service Unit in Oxford for assistance with sample collection and storage.

References

1. Amundadottir LT, Sulem P, Gudmundsson J, et al. A common variant associated with prostate cancer in European and African populations. Nat Genet 2006;38:652–8.
2. Gudmundsson J, Sulem P, Manolescu A, et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nat Genet 2007;39:631–7.
3. Haiman CA, Patterson N, Freedman ML, et al. Multiple regions within 8q24 independently affect risk for prostate cancer. Nat Genet 2007;39:638–44.
4. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087–93.
5. Tomlinson I, Webb E, Carvajal-Carmona L, et al. A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. Nat Genet 2007;39:984–8.
6. Zanke BW, Greenwood CM, Rangrej J, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. Nat Genet 2007;39:989–94.
7. Haiman CA, Le Marchand L, Yamamato J, et al. A common genetic risk factor for colorectal and prostate cancer. Nat Genet 2007;39:954–6.
8. Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007;39:870–4.
9. Marchini J, Donnelly P, Cardon LR. Genome-wide strategies for detecting multiple loci that influence complex diseases. Nat Genet 2005;37:413–7.
10. Goldgar DE, Easton DF, Cannon-Albright LA, et al. Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. J Natl Cancer Inst 1994;86:1600–8.
11. Peto J, Easton DF, Matthews FE, et al. Cancer mortality in relatives of women with breast cancer: the OPCS Study. Office of Population Censuses and Surveys. Int J Cancer 1996;65:275–83.
12. Johnson N, Fletcher O, Naceur-Lombardelli C, et al. Interaction between CHEK2*1100delC and other low-penetrance breast-cancer susceptibility genes: a familial study. Lancet 2005;366:1554–7.
13. Schumacher FR, Feigelson HS, Cox DG, et al. A common 8q24 variant in prostate and breast cancer from a large nested case-control study. Cancer Res 2007;67:2951–6.
14. Fletcher O, Johnson N, Palles C, et al. Inconsistent association between the STK15 F31I genetic polymorphism and breast cancer risk. J Natl Cancer Inst 2006;98:1014–8.
15. Cox A, Dunning AM, Garcia-Closas M, et al. A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet 2007;39:352–8.
16. Breast Cancer Association Consortion. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. J Natl Cancer Inst 2006;98:1382–96.