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Hormone-dependent effects of *FGFR2* and *MAP3K1* in breast cancer susceptibility in a population-based sample of post-menopausal African-American and European-American women

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***FGFR2* and *MAP3K1* are members of the RAS/RAF/MEK/ERK-signaling pathway and have been identified from genome-wide association studies to be breast cancer susceptibility genes. Potential interactions of these genes and their role with respect to tumor markers, hormonal factors and race on breast cancer risk have not been explored. We examined *FGFR2* and *MAP3K1* variants, breast tumor characteristics and hormone exposures in a population-based case–control sample of 1225 European-American (EA) and 584 African-American (AA) women. *FGFR2* rs1219648 and rs2981582 genotypes were significantly associated with breast cancer in EA only in estrogen receptor-positive (ER+), progesterone receptor-positive (PR+) and HER2/Neu-negative (HER2−) tumors. *MAP3K1* was not associated with breast cancer in EA women, but it was associated with breast cancer in AA women, again limited to ER+, PR+ and HER2− tumors. An interaction was observed between combined hormone replacement therapy use and *FGFR2* rs1219648 genotypes on breast cancer risk in EA women ($P = 0.010$). Finally, we observed a significant interaction between *MAP3K1* rs889312 and *FGFR2* rs2981582 ($P = 0.022$) in AA but not EA women. These results confirm that *FGFR2* and *MAP3K1* are involved in breast cancer susceptibility and confer their effects primarily in ER+ and PR+ tumors. We further report that these genes confer their effects in HER2− tumors, interact with one another to confer breast cancer susceptibility in AA women and interact with hormone exposures in AA and EA women.**

Introduction

The *FGFR2* and *MAP3K1* genes were recently identified in two genome-wide association studies (GWAS) of breast cancer (1,2). These genes act in the mitogen-activated protein kinase (MAPK)-signaling pathway that includes RAS, RAF, MEK and ERK, and is responsible for regulation of transcription of important cancer genes including c-Myc, c-Elk1, c-Jun and c-Fos (Figure 1). MAPK signal transduction is a critical pathway for cellular regulation and can be stimulated by a wide variety of exposures including estrogen in a variety of cell types (3,4). *FGFR2* is a member of a receptor tyrosine kinase family of genes that encodes the fibroblast growth factor receptor protein. *FGFR2* is a tumor suppressor gene that is amplified and overexpressed in 10–15% of breast tumors (5,6). *FGFR2* can transform human mammary epithelial cells (6), and inhibition of *FGFR2* signaling can inhibit breast tumor cell proliferation (7). FGFR2 also functions in proliferation and invasion of terminal end buds of the developing breast in mice (8). The most significant single single nucleotide polymorphism (SNP) association in *FGFR2* was estimated by Easton *et al.* in the large multicenter Breast Cancer Association Consortium study to have a per-allele effect of 1.26 [95% confidence interval (CI): 1.23–1.30] and by Hunter *et al.* in the Cancer Genetic Markers of Susceptibility study of 1.32 (1.17–1.49). This association was further confirmed in Israeli populations (9). The *FGFR2* associations reported by Easton *et al.* (1) were found in intron 2, where a number of conserved transcription factor-binding sites were identified, including putative estrogen receptor (ER)-binding sites (10). Finally, *FGFR2* effects have been reported to be relevant in ER- and progesterone receptor (PR)-positive tumors to a greater degree than in ER− or PR− tumors (11,12) and that FGFR2 is differentially expressed in different breast cancer subtypes (13). Subsequently, it was reported that genomic variability in the Oct-1/Runx2- and C/-EBPβ-binding sites of *FGFR2* may represent an explanation for the functional relationship of *FGFR2* expression in breast cancer etiology (14).

MAP3K1 (MEKK1) encodes the MAPK kinase kinase protein that phosphorylates and activates the MAPK kinase (MAPK2) that in turn phosphorylates the MAPK/ERK to produce downstream signaling effects on a variety of cancer genes (Figure 1). The MAPK pathway is strongly linked to HER2 receptor activity, and activating mutations in the MAPK pathway have been associated with HER2+ breast tumors (15,16). MAP3K1 was identified by Easton *et al.* (1) to have a per-allele odds ratio (OR) effect of 1.13 (95% CI: 1.09–1.18). *MAP3K1* effects were found to be relevant in ER+ and PR+ tumors to a greater degree than in ER− or PR− tumors (11). MAP3K1 is differentially expressed in different breast cancer subtypes (13).

Endogenous and exogenous steroid hormone exposures are also strongly associated with breast cancer risk. These exposures include combined hormone replacement therapy (CHRT), reproductive history and obesity (17). However, no studies have explicitly evaluated the role of recent GWAS findings in the context of relevant gene–gene interactions, hormonal exposures and race. Therefore, we assessed whether the role of *FGFR2* and *MAP3K1* involved interactions and race-specific effects on breast cancer risk in a population-based sample of African-American (AA) and European-American (EA) women.

Materials and methods

Study design and data collection

The Women's Insights and Shared Experiences study is a population-based case–control study. Incident breast cancer cases were identified through hospitals and validated via the Pennsylvania State Cancer Registry, and frequency-matched controls were identified from the community using random-digit dialing. The source population for this study was the three counties of Philadelphia (PA), Delaware (PA) and Camden (NJ). Potentially eligible cases were Caucasian and AA women ages 50–79 years old residing in these counties at the time of a new diagnosis of breast cancer between 1 July 1999 and 30 June 2002. Controls were selected from the same geographic regions as the cases and were frequency matched to the cases on race and age (in 5-year age groups). The present analysis thus involved 528 EA breast cancer cases and 697 age-matched controls and 157 AA breast cancer cases and 427 age-matched controls. We used picture card reminders of medications that the woman may have used, as well as a lifetime calendar of reproductive events to assess exogenous and endogenous hormone exposures. Medical record abstractions identified tumor characteristics including tumor marker status. Genomic DNA was obtained from buccal swabs as described previously (18). Additional details of our study design have been previously reported (18–21).

The study was approved by the University of Pennsylvania Committee on Studies Involving Human Beings and by the Institutional Review Boards of all

Abbreviations: AA, African-American; CHRT, combined hormone replacement therapy; CI, confidence interval; EA, European-American; ER, estrogen receptor; GWAS, genome-wide association study; MAPK, mitogen-activated protein kinase; OR, odds ratio; PR, progesterone receptor; SNP, single nucleotide polymorphism.

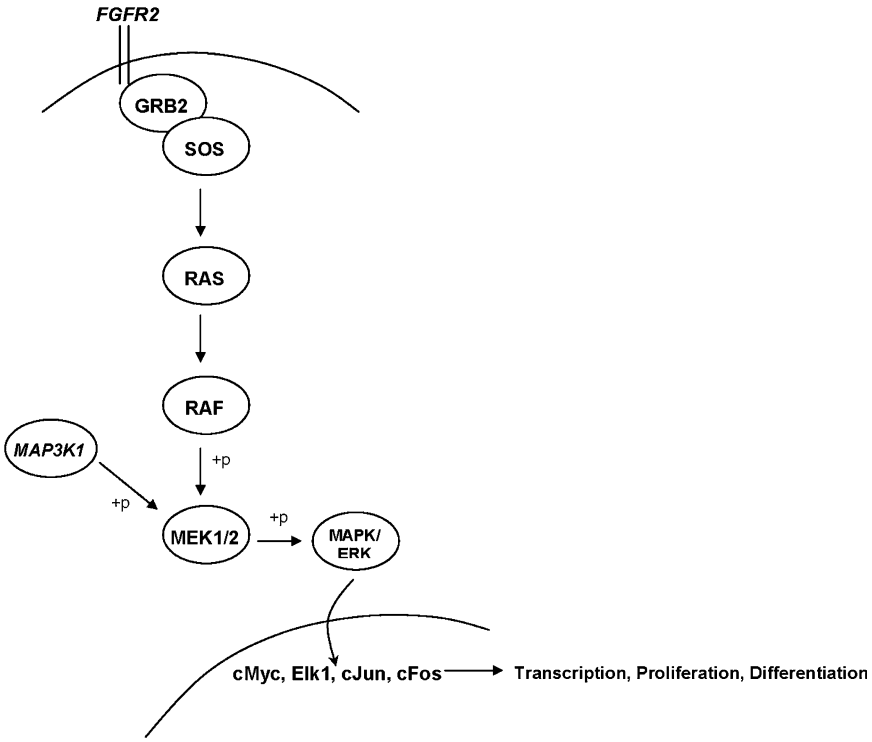

Fig. 1. The MAPK transduction signaling pathway.

the participating hospitals. Participants provided verbal informed consent for the interview and written informed consent for the buccal samples.

Laboratory methods

We typed three SNPs in the MAPK-signaling pathways that were previously reported as having genome-wide significance in two GWAS: *FGFR2* IVS2 + 7033 rs1219648, the top *FGFR2* hit in the study of Hunter *et al.* (2); *FGFR2* IVS2 + 906 rs2981582, the top *FGFR2* hit in the study of Easton *et al.* (1) and *MAP3K1* rs8893120 reported in the study of Easton *et al.* (1).

Samples were genotyped using the ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA) using the standard protocol. Briefly, DNA was extracted from buccal swabs on a Qiagen 9604B Biorobot using a QIAamp 96 DNA Swab BioRobot Kit (Qiagen, Valencia, CA). Polymerase chain reaction for each SNP was performed using 5 PRIME MasterMix (5 Prime5, Gaithersburg, MD) in three separate multiplex reactions with other SNPs in the study. Polymerase chain reactions were then cleaned with ExoSAP. Multiplexed single base extension reactions were performed with base extension primer primers to label the alleles present with the appropriate Fluorescein-labeled dideorynucleotides and then cleaned with SAP. These were then run on an ABI PRISM 3100 Genetic Analyzer (ABI, Foster City, CA). Standardized quality control measures were used that included positive and negative (water) controls on each plate, repeat samples to ensure minimal discordance across samples, low failure rates (<20%) and no deviation from Hardy–Weinberg proportions. Genotypes were read using GeneMapper 4.0.

Statistical methods

OR estimates and 95% CIs were calculated to evaluate the relationship between genes found in GWAS and hormone exposures and tumor markers with breast cancer risk. Multiple conditional logistic regression was used to account simultaneously for the matching variables (defined by combinations of age group and interview date) and known risk factors for breast cancer. A variable was considered a confounder if it changed the point estimate of any genotype effect by 10% or more. All models considered the same set of confounders: number of full-term pregnancies (0, 1, 2 and 3+) and age at menopause.

We tested for interaction between each genetic variant and endogenous or exogenous hormone exposures that are known breast cancer risk factors using a likelihood ratio chi-square test. We undertook a 2 df test of interaction across three-level genotype (assuming the existence of a linear relationship by coding genotypes 0, 1 and 2) and exposure as well as a 1 df test of the per-allele (trend) effect of genotypes. We also stratified the analyses by tumor markers ER, PR and HER2 status. For comparisons stratified by tumor characteristics, the total set of eligible, genotyped controls were compared with the cases in each tumor characteristics stratum. All *P*-values are based on two-sided hypothesis tests. All analyses were performed in STATA (version 9.0, STATA Corporation, College Station, TX).

Results

Table I presents the descriptive characteristics of the study sample. The allele frequencies for each locus were similar to those previously reported in EA populations (1,2) and are comparable with those found in public databases for AA populations. All variants were consistent with Hardy–Weinberg proportions in controls of both races. Tumor marker characteristics differed as expected between AA and EA, with AA having a lower proportion of ER+, PR+ and HER2+ tumors.

Consistent with previous reports, we observed a significant association between *FGFR2* genotypes and breast cancer in EA post-menopausal women (Table II), with per-allele effects of 1.23 (95% CI: 1.03–1.46) for rs1219648 and 1.26 (95% CI: 1.04–1.53) for rs2981582. We also report that the effect of these genotypes appears to be limited to ER+ tumors, PR+ tumors and HER2− tumors. In every case, the per-allele effects of *FGFR2* ranged from 1.30 to 1.43. In ER−, PR− or HER2+ tumors, per-allele effects were very near OR = 1.0. We did not observe a significant association with *MAP3K1* in EA post-menopausal women.

In contrast to the results in EA post-menopausal women, we observed no effect of *FGFR2* in AA post-menopausal women (Table III). None of the per-allele OR effects for the two *FGFR2* SNPs was >1.2, and many of the estimates were OR <1.0. For *MAP3K1*, we observed no significant association overall. However, we report statistically significant associations of *MAP3K1* rs889312 genotypes in ER+, PR+ or HER2− tumors (Table III). In each case, the per-allele OR effect was approximately OR = 1.5, with homozygote CC genotype effects in the range of ∼2.4–2.8.

We also considered the joint effects of *FGFR2* or *MAP3K1* genotypes and hormone-related breast cancer risk factors (Tables IV and V). *FGFR2* rs1219648 genotypes interacted significantly with the use of CHRT (*P*-value = 0.010). Breast cancer risk was elevated among never users of CHRT who carried any *FGFR2* rs1219648 genotype or if they had used CHRT but carried the AA genotype. For *FGFR2*

Table I. Descriptive characteristics of 584 AA and 1225 EA participants in the Women's Insights and Shared Experiences study

Variable	AA cases (n = 157)	AA controls (n = 427)	EA cases (n = 528)	EA controls (n = 697)
FGFR2 IVS2 + 7033 (rs1219648) G allele frequency (HWE *P*-value)	0.42 (P = 0.283)	0.45 (P = 0.216)	0.47 (P = 0.247)	0.42 (P = 0.227)
FGFR2 IVS2 + 906 (rs2981582) T allele frequency (HWE *P*-value)	0.46 (P = 0.415)	0.52 (P = 0.792)	0.47 (P = 0.304)	0.43 (P = 0.765)
MAP3K1 (rs889312) C allele frequency (HWE *P*-value)	0.38 (P = 0.636)	0.30 (P = 0.916)	0.31 (P = 0.012)	0.29 (P = 0155)
Proportion ER+	57%	—	81%	—
Proportion PR+	61%	—	70%	—
Proportion HER2+	39%	—	36%	—
Mean age at diagnosis (cases) or interview (controls) (SD)	62.0 (7.8)	60.6 (7.8)	63.4 (8.7)	62.5 (8.3)
Mean number of full-term pregnancies (SD)	3.4 (2.4)	3.7 (2.4)	2.8 (2.1)	3.2 (2.1)
Mean age at menarche (SD)	12.7 (2.4)	12.7 (1.8)	12.5 (1.5)	12.7 (1.7)
Mean age at menopause (SD)	46.2 (7.2)	45.8 (7.7)	47.8 (5.9)	47.9 (6.1)

SD, standard deviation.

Table II. Adjusted ORs with 95% CIs[a] for the effect of genotypes on breast cancers: 1225 EA women

Locus	Genotype	Total	ER+	ER−	PR+	PR−	HER2+	HER2−
FGFR2 IVS2 + 7033 (rs1219648)	AA	(1)	(1)	(1)	(1)	(1)	(1)	(1)
	AG	1.18 (0.89–1.57)	1.48 (1.06–2.05)	0.63 (0.36–1.09)	1.55 (1.09–2.20)	0.82 (0.53–1.280	1.05 (0.66–1.65)	1.53 (1.06–2.22)
	GG	1.52 (1.08–2.14)	1.68 (1.13–2.52)	0.95 (0.49–1.82)	1.84 (1.20–2.81)	1.01 (0.59–1.75)	1.05 (0.58–1.90)	1.71 (1.09–2.69)
	Per G allele	1.23 (1.03–1.46)	1.31 (1.07–1.60)	0.91 (0.65–1.28)	1.37 (1.11–1.69)	0.98 (0.74–1.29)	1.03 (0.77–1.37)	1.32 (1.06–1.65)
FGFR2 IVS2 + 906 (rs2981582)	CC	(1)	(1)	(1)	(1)	(1)	(1)	(1)
	CT	1.17 (0.85–1.61)	1.37 (0.95–2.00)	0.91 (0.49–1.69)	1.47 (0.99–2.20)	0.97 (0.59–1.59)	1.13 (0.67–1.90)	1.44 (0.95–2.18)
	TT	1.62 (1.10–2.39)	1.87 (1.19–2.95)	1.01 (0.46–2.24)	2.05 (1.26–3.32)	1.11 (0.59–2.09)	1.19 (0.60–2.35)	1.68 (1.00–2.80)
	Per T allele	1.26 (1.04–1.53)	1.37 (1.09–1.71)	0.99 (0.67–1.47)	1.43 (1.13–1.82)	1.04 (0.76–1.43)	1.10 (0.79–1.53)	1.30 (1.01–1.68)
MAP3K1 rs889312	AA	(1)	(1)	(1)	(1)	(1)	(1)	(1)
	AC	1.02 (0.76–1.36)	1.18 (0.84–1.64)	0.81 (0.46–1.42)	1.06 (0.75–1.51)	1.14 (0.73–1.80)	0.90 (0.56–1.45)	1.17 (0.81–1.69)
	CC	1.32 (0.84–2.08)	1.50 (0.90–2.49)	0.30 (0.07–1.30)	1.44 (0.84–2.49)	0.79 (0.35–1.78)	1.00 (0.47–2.14)	1.26 (0.70–2.26)
	Per C allele	1.10 (0.90–1.35)	1.21 (0.96–1.52)	0.69 (0.44–1.08)	1.15 (0.90–1.47)	0.98 (0.71–1.36)	0.96 (0.69–1.35)	1.14 (0.88–1.47)

[a]Estimated from conditional logistic regression adjusted for age at menopause, number of full-term pregnancies and matched on age.

Table III. Adjusted ORs with 95% CIs[a] for the effect of genotypes on breast cancers: 584 AA women

Locus	Genotype	Total	ER+	ER−	PR+	PR−	HER2+	HER2−
FGFR2 IVS2 + 7033 (rs1219648)	AA	(1)	(1)	(1)	(1)	(1)	(1)	(1)
	AG	1.04 (0.68–1.58)	0.93 (0.55–1.58)	1.48 (0.73–3.00)	1.18 (0.68–2.06)	0.99 (0.52–1.86)	1.43 (0.69–2.98)	1.02 (0.59–1.75)
	GG	0.64 (0.37–1.13)	0.81 (0.41–1.59)	0.86 (0.56–1.33)	0.65 (0.30–1.42)	0.88 (0.40–1.92)	0.82 (0.31–2.20)	0.84 (0.42–1.66)
	Per G allele	0.84 (0.64–1.09)	0.90 (0.65–1.26)	1.18 (0.68–2.06)	0.87 (0.61–1.23)	0.94 (0.64–1.38)	0.96 (0.62–1.49)	0.93 (0.66–1.29)
FGFR2 IVS2 + 906 (rs2981582)	CC	(1)	(1)	(1)	(1)	(1)	(1)	(1)
	CT	0.79 (0.48–1.32)	0.88 (0.47–1.64)	1.06 (0.42–2.66)	0.88 (0.46–1.68)	1.12 (0.49–2.58)	0.64 (0.28–1.43)	1.38 (0.69–2.76)
	TT	0.64 (0.35–1.16)	0.81 (0.40–1.66)	0.55 (0.17–1.79)	0.67 (0.31–1.45)	1.01 (0.39–2.59)	0.60 (0.28–1.43)	1.11 (0.50–2.48)
	Per T allele	0.80 (0.59–1.08)	0.90 (0.63–1.29)	0.77 (0.44–1.32)	0.82 (0.56–1.20)	1.00 (0.63–1.59)	0.76 (0.47–1.23)	1.05 (0.71–1.53)
MAP3K1 rs889312	AA	(1)	(1)	(1)	(1)	(1)	(1)	(1)
	AC	1.24 (0.79–1.94)	1.22 (0.70–2.15)	1.12 (0.52–2.39)	1.02 (0.56–1.87)	1.44 (0.73–2.84)	0.87 (0.41–1.85)	1.51 (0.84–2.71)
	CC	1.86 (0.96–3.60)	2.84 (1.32–6.09)	1.28 (0.38–4.25)	2.85 (1.29–6.28)	1.52 (0.54–4.29)	2.12 (0.8–5.48)	2.40 (1.03–5.57)
	Per C allele	1.33 (0.98–1.81)	1.57 (1.08–2.29)	1.13 (0.66–1.93)	1.52 (1.02–2.26)	1.29 (0.81–2.05)	1.31 (0.80–2.13)	1.54 (1.03–2.29)

[a]Estimated from conditional logistic regression adjusted for age at menopause, number of full-term pregnancies and matched on age.

rs2981582, we also observed a significant increase in breast cancer risk among women who had never used CHRT and had the rs2981582 TT genotype (OR = 1.87, 95% CI: 1.08–3.22). Nulliparous women with the *FGFR2* rs1219648 GG genotype were at significantly increased breast cancer risk (OR = 4.04, 95% CI: 1.26–12.98), whereas no excess risk was observed for any other genotype- or parity-specific group. Risk was also increased among nulliparous women with the rs2981582 TT genotype (OR = 9.68, 95% CI: 1.90–49.31).

No statistically significant interactions were observed among AA women (Table V). However, we observed two stratum-specific effects. First, we report an inverse relationship with breast cancer risk among women who carried the *MAP3K1* AA genotype and who had a later age at menarche (OR = 0.44, 95% CI: 0.21–0.93). Second, we report an inverse relationship between women who carried the *FGFR2* rs1219648 AA genotype and were parous (OR = 0.31, 95% CI: 0.10–0.94).

Finally, because these two genes are both involved in the MAPK signal transduction pathway, we evaluated the potential for gene–gene interaction effects. Among EA women, we observed no statistically significant interactions for carriage of any variant for *MAP3K1* rs889312 and either *FGFR2* rs1219648 or rs2981582 (*P*-value for

Table IV. Adjusted ORs with 95% CIs[a] for the effect of genotypes on breast cancers in 1225 EA women by hormonal exposures

Locus	Genotype	No CHRT	Any CHRT	Interaction *P*-value	Nulliparous	Parous	Interaction *P*-value	Menarche <age 12	Menarche age 12+	Interaction *P*-value
FGFR2 IVS2 + 7033	AA	(1)	2.63 (1.46–4.76)	0.010	(1)	0.58 (0.30–1.12)	0.191	(1)	0.95 (0.55–1.62)	0.660
(rs1219648)	AG	1.53 (1.04–2.26)	0.49 (0.26–0.95)		1.46 (0.68–3.13)	1.15 (0.86–1.53)		1.52 (0.82–2.82)	1.11 (0.81–1.52)	
	GG	1.59 (0.99–2.55)	1.12 (0.51–2.44)		4.04 (1.26–12.98)	1.31 (0.92–1.86)		1.86 (0.91–3.78)	1.43 (0.97–2.13)	
FGFR2 IVS2 + 906	CC	(1)	1.76 (0.87–3.52)	0.261	(1)	0.66 (0.31–1.39)	0.061	(1)	0.96 (0.53–1.75)	0.716
(rs2981582)	CT	1.46 (0.94–2.27)	0.73 (0.34–1.54)		1.65 (0.70–3.95)	1.06 (0.77–1.46)		1.50 (0.76–2.95)	1.09 (0.76–1.57)	
	TT	1.87 (1.08–3.22)	1.55 (0.64–3.80)		9.68 (1.90–49.31)	1.28 (0.87–1.90)		1.88 (0.84–4.21)	1.55 (0.99–2.43)	
MAP3K1 rs889312	AA	(1)	1.04 (0.62–1.75)	0.431	(1)	0.34 (0.19–0.61)	0.641	(1)	0.67 (0.43–1.04)	0.443
	AC	0.94 (0.63–1.39)	1.15 (0.58–2.28)		0.77 (0.34–1.75)	1.04 (0.78–1.40)		0.75 (0.41–1.38)	1.11 (0.80–1.54)	
	CC	1.01 (0.52–1.98)	2.13 (0.86–5.24)		0.79 (0.22–2.84)	1.40 (0.89–2.20)		1.50 (0.59–3.86)	1.27 (0.76–2.14)	

[a]Estimated from conditional logistic regression adjusted for age at menopause, number of full-term pregnancies and matched on age.

Table V. Adjusted ORs with 95% CIs[a] for the effect of genotypes on breast cancers in 584 AA women by hormonal exposures

Locus	Genotype	No CHRT	Any CHRT	Interaction *P*-value	Nulliparous	Parous	Interaction *P*-value	Menarche <age 12	Menarche age 12+	Interaction *P*-value
FGFR2 IVS2 + 7033	AA	(1)	1.13 (0.20–6.19)	0.890	(1)	0.31 (0.10–0.94)	0.416	(1)	0.95 (0.45–2.00)	0.395
(rs1219648)	AG	1.12 (0.66–1.90)	1.67 (0.25–11.22)		0.49 (0.13–1.90)	1.22 (0.80–1.86)		1.41 (0.62–3.22)	0.93 (0.57–1.52)	
	GG	0.83 (0.40–1.71)	0.79 (0.05–13.20)		0.80 (0.04–15.78)	0.76 (0.44–1.32)		0.45 (0.13–1.57)	0.70 (0.37–1.32)	
FGFR2 IVS2 + 906	CC	(1)	1.60 (0.24–10.75)	0.932	(1)	0.82 (0.18–3.71)	0.608	(1)	0.80 (0.34–1.88)	0.750
(rs2981582)	CT	0.93 (0.49–1.77)	0.82 (0.10–6.99)		1.81 (0.3–10.80)	0.71 (0.43–1.19)		0.95 (0.37–2.43)	0.74 (0.41–1.36)	
	TT	0.99 (0.46–2.11)	0.55 (0.03–11.48)		1.07 (0.11–10.71)	0.69 (0.38–1.25)		0.51 (0.15–1.77)	0.67 (0.33–1.34)	
MAP3K1 rs889312	AA	(1)	1.32 (0.41–4.27)	0.923	(1)	0.42 (0.12–1.45)	0.953	(1)	0.44 (0.21–0.93)	0.124
	AC	1.37 (0.78–2.44)	1.09 (0.14–8.41)		1.08 (0.22–5.23)	1.27 (0.81–1.98)		0.52 (0.20–1.35)	1.60 (0.96–2.68)	
	CC	1.78 (0.80–3.95)	1.06 (0.08–13.39)		1.33 (0.13–13.56)	1.93 (0.99–3.73)		1.33 (0.41–4.28)	2.01 (0.89–4.52)	

[a]Estimated from conditional logistic regression adjusted for age at menopause, number of full-term pregnancies and matched on age.

interaction: 0.376 and 0.569, respectively). In AA women, we found no statistically significant interactions for carriage of any variant for *MAP3K1* rs889312 and *FGFR2* rs1219648 (*P*-value for interaction: 0.464). However, we did observe a significant interaction between *MAP3K1* rs889312 and *FGFR2* rs2981582 (*P*-value for interaction: 0.022). Specifically, we observed that the highest risk group was in AA women who carried any MAPK31 AC or CC genotypes and the *FGFR2* rs2981582 CC genotype (OR = 3.84, 95% CI: 1.83–8.04). This interaction is consistent with the main effects for each of these genotypes shown in Table III.

Discussion

We have identified significant associations of *FGFR2* and *MAP3K1* that confer breast cancer risk in a steroid hormone-dependent manner. We also have observed an interaction of *MAP3K1* and *FGFR2* in AA women. These results confirm and extend the previous GWAS reports that these two genes are breast cancer susceptibility genes.

Our data indicate that the association among population-based breast cancer cases is strongest in ER+, PR+ or HER2− breast tumors. These results are most consistent with the possibility that *FGFR2* and *MAP3K1* affect breast cancer etiology in a steroid hormone-dependent manner. These hormone-dependent associations are biologically plausible. *MAP3K1* is a serine/threonine kinase that plays a central role in the MAPK cascade of phosphorylating enzymes that responds to a number of mitogenic and metabolic stimuli, including estrogen (3,4). *FGFR2* is amplified and overexpressed in 10–15% of breast tumors (5,6), can transform human mammary epithelial cells (6), and inhibition of *FGFR2* signaling can inhibit breast tumor cell proliferation (7). *FGFR2* contains at least one putative ER-binding site (1). Therefore, it is biologically plausible that hormone exposures in combination with genomic variability in MAPK signal transduction pathways may influence breast cancer susceptibility.

In addition to ER and PR status, the MAPK pathway is strongly linked to HER2 receptor activity, and activating mutations in the MAPK pathway have been associated with HER2+ breast tumors (15,16). Thus, MAPK signaling should be correlated with HER2-positive tumors. We report that mutations in FGFR2 or MAP3K are associated with HER2− tumors as would be predicted if these mutations impede HER2-associated signaling through the MAPK pathway.

We also identified a statistically significant interaction between *FGFR2* rs1219648 and CHRT on breast cancer risk. CHRT containing both estrogens and progestins has been widely used to treat menopausal symptoms (22). Serious concerns have been raised about adverse effects of CHRT use, including risks of pulmonary embolism (23–26), stroke (27), coronary heart disease (28) and cancer (29–33). While CHRT influences breast cancer risk, the excess risk conferred by CHRT may not be the same in all users. There is substantial evidence that genetic variants in candidate hormone metabolism genes may influence the disposition of exogenous hormones found in CHRT. For example, variability in hormone metabolism determined by inherited genotypes may influence breast tumorigenesis (34,35). Our data are consistent with the hypothesis that subsets of the population may have different susceptibility to the effects of CHRT due to interindividual variation in the *FGFR2* gene. Thus, *FGFR2* genotypes may influence whether women exposed to CHRT are differently susceptible to the breast carcinogenic effects of these compounds.

While our results are biologically plausible, there are a number of limitations to this research. While our study was designed to detect first-order interactions, the power to detect first-order interactions with small magnitudes was low. Of great importance is an understanding of the relationship of genotype by hormone exposure interactions within race and by ER/PR status. Analyses that simultaneously undertake stratified analyses of relevant interaction effects will require much larger samples than were possible in the present study. However, very small effects may also not be clinically relevant even though they may be biologically important, so the impact of our research may be to identify effects that may ultimately have clinical utility. While we chose SNPs that were previously reported to be important from GWAS results, only a limited number of SNPs were tested here, and it is probably that the SNPs that are causative of breast cancer were not included here. Similarly, while some information about the function of the SNPs studied here is known, additional functional data are required before the associations we report can be fully interpreted. Despite these limitations, the present study has a number of strengths, sufficient power was available to detect stratum-specific and interaction effects in both AA and EA women in this population-based study.

In addition to the important etiological implications of these findings, there are also clinical implications of the discovery that these genes are involved in breast cancer etiology. Breast cancer treatment and chemoprevention strategies have been explored that target the MAPK pathway, including those that involve inhibition of RAF kinase such as sorafenib (36). Receptor tyrosine kinases have already proven to be successful targets for the development of therapies such as Herceptin and Gleevec.

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