Published in final edited form as:

Breast Cancer Res Treat. 2014 September ; 147(2): 381–387. doi:10.1007/s10549-014-3081-9.

Association between germline single nucleotide polymorphisms in the PI3K-AKT-mTOR pathway, obesity, and breast cancer disease free survival

Mala Pande¹, Melissa L. Bondy², Kim-Anh Do³, Aysegul A. Sahin⁴, Jun Ying³, Gordon B. Mills⁵, Patricia A. Thompson⁶, and Abenaa M. Brewster⁷

¹Department of Gastroenterology – Research, The University of Texas MD Anderson Cancer Center, Houston Texas 77030, USA

²Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, Texas 77030, USA

³Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston Texas 77030, USA

⁴Department of Pathology, Systems, The University of Texas MD Anderson Cancer Center, Houston Texas 77030, USA

⁵Department of Biology, The University of Texas MD Anderson Cancer Center, Houston Texas 77030, USA

⁶Department of Cellular and Molecular Medicine, The University of Arizona Cancer Center, Tucson, Arizona 85719, USA

⁷Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston Texas 77030, USA

Abstract

Purpose—Obesity-related hormones and cytokines alter PI3K-AKT-mTOR pathway activation in breast tumors contributing to poorer disease-free survival (DFS) and decreased responsiveness to tamoxifen and trastuzumab. We hypothesized that single nucleotide polymorphisms (SNPs) in candidate genes in the PI3K-AKT-mTOR signaling pathway may act as genetic modifiers of breast cancer DFS.

Methods—We analyzed the association of 106 tagging SNPs in 13 genes (*ADIPOQ*, *IGF1*, *INS*, *IRS1*, *LEP*, *LEPR*, *LEPROT*, *PIK3CA*, *PIK3R5*, *PTEN*, *TSC1*, *TSC2*, and *AKT1*) in the PI3K-AKT-mTOR pathway with DFS in a sample of 1,019 women with stage I–II breast cancer. SNPs significantly associated with DFS in any genetic model (additive, dominant or recessive) after

To whom correspondence should be addressed. Abenaa M. Brewster, The University of Texas MD Anderson Cancer Center, Department of Clinical Cancer Prevention, Unit 1360, 1515 Holcombe Blvd., Houston, TX 77030. Tel: 7137454928; Fax: 7135635746; abrewster@mdanderson.org.

Conflict of Interest Statement: Dr. Gordon Mills has served as a consultant for AstraZeneca, Blend, Critical Outcome Technologies, HanAI Bio Korena, Illumina, Nuevolution, Pfizer, Provista Diagnostics, Roche, Signalchem Lifesciences, Symphogen and Tau Therapeutics. He has ownership interests in Catena Pharmaceuticals, PTV Ventures, Spindle Top Ventures and has received commercial research grants from Adelson Medical Research Foundation, AstraZeneca, Critical Outcomes Technology and GlaxoSmithKline.

correcting for false discovery rate (FDR=0.10) were included in Cox proportional hazards multivariable analyses.

Results—After adjusting for race/ethnicity, age at diagnosis, tumor stage and treatment, rs1063539 in *ADIPOQ*, rs11585329 in *LEPR*, and rs2519757 in *TSC1* were associated with improved DFS and rs1520220 in *IGF1* and rs2677760 in *PIK3CA* were associated with worse DFS. The associations were not significantly modified by type of systemic treatment received or body mass index. The SNPs were not associated with tumor characteristics such as tumor size, lymph node status, nuclear grade, or hormone receptor status.

Conclusion—In this study, germline SNPs in the PI3K-AKT-mTOR pathway were associated with breast cancer DFS and may be potential prognostic markers. Future studies are needed to replicate our results and to evaluate the relationship between these polymorphisms and activation of the PI3K-AKT-mTOR pathway in breast tumors.

Keywords

Breast cancer; prognosis; PI3K-AKT-mTOR pathway; single nucleotide polymorphisms; disease-free survival

Background

Obesity is an established risk factor for postmenopausal breast cancer and expanding epidemiological evidence suggests that obesity is independently associated with breast cancer recurrence and survival regardless of menopausal status [1,2]. Although the modifying effect of obesity on prognostic breast tumor characteristics has been studied extensively [3,4], there are limitations in our understanding of the contribution of genes involved in obesity-related signaling pathways to breast cancer outcomes.

Growth factors (e.g. IGF-1) and obesity-related hormones and cytokines (e.g. insulin, adiponectin) interact with mammalian target of rapamycin (mTOR) through activation of the phosphatidylinositor-3 kinase (PI3K)-AKT pathway [5,6]. Activation of mTORC1 either through upstream insulin or growth factor receptor signaling or genetic/epigenetic abnormalities promotes cell proliferation and inhibits apoptosis through the phosphorylation of downstream targets including p70S6K, and subsequently 4E-binding proteins (4E-BPs), and ribosomal protein S6 kinase 1 (S6K1) resulting in enhanced protein translation [5,6]. PI3K-AKT-mTOR pathway activation has been associated with decreased responsiveness to a wide range of breast cancer therapies including tamoxifen, aromatase inhibitors and trastuzumab [7–14]. Recently, the mTOR inhibitor everolimus was approved in the U.S for the treatment of metastatic breast cancer resistant to aromatase inhibitors [7].

Despite the importance of the PI3K-AKT-mTOR pathway in breast cancer and the link with obesity, there are no studies to date that have evaluated the role of germline variations in the PI3K-AKT-mTOR pathway and breast cancer outcomes. Prior epidemiological studies have shown significant associations between single nucleotide polymorphisms (SNPs) of candidate genes in the PI3K-AKT-mTOR pathway and increased risk of death from bladder cancer [15,16] and recurrence of esophageal cancer [17]. Promising candidates for investigation include obesity related genes involved in the initiation of pathway activation

such as *INS*, *IGF1*, *IRS1*, *ADIPOQ* and genes that represent the core functional components of the pathway such as *PIK3CA*, *mTOR*, *AKT* and the tumor suppressor gene, *PTEN*. This study utilizes a well characterized cohort of patients with early stage breast cancer with detailed information on tumor characteristics and treatment to determine whether tagging SNPs selected for each of the candidate genes in the PI3K-AKT-mTOR pathway are associated with breast cancer disease-free survival (DFS).

Methods

Study population

The Early Stage Breast Cancer Repository (ESBCR) is a retrospective cohort of 2,409 women diagnosed with American Joint Committee on Cancer pathologic stage I or II breast cancer and surgically treated at The University of Texas MD Anderson Cancer Center between 1985 and 2000. Criteria for eligibility and cohort details have been previously described [18]. Briefly, we selected a subset of the ESBCR population with available formalin-fixed paraffin-embedded normal lymph nodes or blood samples available for DNA extraction for participation in the genotyping project. Detailed clinical information including patient age, race/ethnicity, stage, tumor size, lymph node status, nuclear grade, estrogen receptor (ER) and progesterone receptor (PR) status, and primary treatment (including surgery, radiation therapy, chemotherapy and endocrine therapy) were abstracted from medical charts. Follow-up information for patients in the ESBCR is obtained by direct review of the medical records to determine dates of relapse or death, and linkage to the MD Anderson Cancer Center Tumor Registry, which mails annual follow-up letters to each patient registered at MD Anderson Cancer Center known to be alive to determine their clinical status. The MD Anderson Tumor Registry checks the social security death index and the Texas Bureau of Vital Statistics for the status of patients that fail to respond to letters. We selected a subset of the ESBCR population for participation in the genotyping project enriched to include all African American (n=196) and Hispanic patients (n=208) and a random sample of non-Hispanic White patients (n=986). Of these, 301 patients were excluded because of DNA extraction failure, 54 because of genotype assay failure and 6 because of inadequate clinical information. The final study analysis included 1029 breast cancer patients.

SNP selection and genotyping

We reviewed the literature and selected the following candidate genes of interest in the PI3K-AKT-mTOR pathway: *ADIPOQ*, *IGF1*, *INS*, *IRS1*, *LEP*, *LEPR*, *LEPROT*, *PIK3CA*, *PIK3R5*, *PTEN*, *TSC1*, *TSC2* and *AKT1*. Tagging SNPs were selected within and 2-kb flanking each gene using an algorithm by Carlson et al [19] based on data from the HapMap project (www.hapmap.org Version 23) [20]. All selected SNPs met the following criteria: minor allele frequency (MAF) of 0.05, Illumina design score >0.04 and $r^2 > 0.8$ for binning. Genotyping of the breast cancer cases was performed using Illumina GoldenGate technology (Illumina, San Diego, CA) as part of a larger array of 1,514 SNPs in total. Briefly, genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Maxi kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol [21]. Genomic DNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue using the Pico

Pure DNA extraction kit [22]. The success call rates for the FFPE and non-FFPE samples were 92% and 99% respectively. Blinded duplicate samples (5%) were included in the array platform and the reproducibility rate was 100%. All genotyping information was analyzed and exported using the GenomeStudio software (Illumina).

Statistical methods

Principal component analysis was performed for detecting population structure and potential outliers within each ethnic group (Golden Helix SNP and Variation Suite, Golden Helix Inc, Bozeman, MT). SNPs with genotype missing in >10% of individuals, or with MAF <0.05 were also excluded. Breast cancer DFS was calculated as the time from the date of first treatment to the date of breast cancer recurrence or date of death due to breast cancer, whichever came first.

To evaluate the relationship between each SNP and DFS, univariate Cox proportional hazard regression analysis was performed using three genetic models: additive, dominant and recessive. Correction for multiple testing was performed on univariate SNP analysis results using Benjamini-Hochberg FDR method [23]. SNPs with Wald statistic Chi-square $p < 0.1$ in univariate analyses for any of the 3 models were selected and pairwise linkage disequilibrium (LD) test was performed. SNPs with $R^2 > 0.8$ were excluded. Remaining SNPs were used as input variables for multi-SNP analysis. For each SNP, the model (additive, dominant or recessive) with the lowest p-value was used. Stepwise multivariable Cox regression analyses were performed for clinical/phenotypic variables race/ethnicity, age of diagnosis, nuclear grade, presence of ER or PR, stage, treatment (endocrine therapy) and body mass index (BMI). We categorized body mass index (BMI) based on the cut points for normal weight ($BMI < 25 \text{ mg/kg}^2$) and overweight or obese ($BMI \geq 25 \text{ mg/kg}^2$). Variables with Wald statistics $p < 0.05$ in the multivariable model were selected as covariates for inclusion in the multivariable model along with SNPs described above. Race was not significant at Wald $p < 0.05$ but was included in the model to account for any residual confounding. Stepwise analysis was performed using all individuals in the dataset as discovery set. Threshold for significance for SNPs in the multivariable model was $p < 0.05$. Multivariable analyses were also performed stratified to evaluate the effect of individual SNPs by race and BMI. Associations were quantified using hazard ratios (HRs) and 95% confidence intervals (CIs). Kaplan-Meier analysis was performed to estimate survival over time and test homogeneity between strata within a SNP. We used SAS version 9.1 (SAS Institute, Cary, NC) and R (<http://www.r-project.org>) to perform all of our analyses.

Results

From the subset of 1029 ESBCR patients an additional ten outliers (2 white, 6 African-American and 2 Hispanic patients) were excluded based on principal component analysis resulting in 1019 women in the final analyses. Of these 72% were non-Hispanic white, 13% were African American, 11% were Hispanic and 3% were of unknown race/ethnicity. Demographic and clinical characteristics of the study population and the univariate associations with breast cancer DFS are listed in Table 1. One hundred six tagging SNPs in 13 genes related to the PI3K-AKT-mTOR pathway were analyzed. Of these 106 SNPs, 27

SNPs significantly associated with increased or decreased DFS in any genetic model (additive, dominant or recessive) were selected with $p < 0.1$ in univariate Cox regression analysis. Only 1 SNP out of the 27 was excluded due to high LD ($R^2 > 0.8$) (rs5742629 rs7956547 had LD=0.84, and SNP rs5742629 was excluded), leaving 26 SNPs for inclusion in multivariable analyses. In the univariate model, age at diagnosis, tumor stage and treatment were identified as significant clinical covariates for breast cancer DFS (Table 1). After adjusting for these covariates, as well as for race/ethnicity, 5 of the 26 SNPs were significantly associated with breast cancer DFS ($p < 0.05$) (Table 2). SNPs rs1063539 in *ADIPOQ* (HR = 0.40, 95% CI = 0.19 – 0.86), rs11585329 in *LEPR* (HR = 0.72, 95% CI = 0.53 – 0.98), and rs2519757 in *TSC1* (HR = 0.29, 95% CI = 0.09 – 0.91) were associated with improved DFS and rs1520220 in *IGF1* (HR = 1.56, 95% CI = 1.21 – 2.00) and rs2677760 in *PIK3CA* (HR = 1.43, 95% CI = 1.07 – 1.92) were associated with worse DFS. The associations were not modified by type of systemic treatment received and the individual SNPs were also not associated with tumor characteristics such as tumor size, lymph node status, nuclear grade, or hormone receptor status. Kaplan-Meier analysis for breast cancer DFS by *IGF1* rs1520220 (the SNP with the smallest association p-value), indicated a statistically significant difference (log-rank test p-value=0.0004) between the homozygous wild-type genotype (WW) and the heterozygous and homozygous variant genotypes (WM/MM) which were the genotypes associated with worse DFS (Fig. 3). In analysis stratified by race/ethnicity, 4 out of the 5 SNPs were significantly associated with breast cancer DFS among Whites. None of the 5 SNPs were statistically significant among Black (n=135) or Hispanic patients (n=115), however the sample size for these groups was potentially too small to provide power to detect associations.

In the analysis stratified by BMI ($< 25 \text{ mg/kg}^2$ and $\geq 25 \text{ mg/kg}^2$) (Table 3), The strength of the association between SNP rs1063539 in *ADIPOQ* and improved DFS was increased in patients with normal (HR 0.33; 95% CI 0.12–0.90) compared to overweight or obese status (HR 0.63; 95% CI 0.20–2.01). On the contrary, the strength of the association between rs1520220 in *IGF1* and rs2677760 in *PIK3CA* and worse DFS was statistically significantly increased in the overweight and obese group but not in those with normal weight (Table 3). None of the interaction terms were statistically significant ($p > 0.05$).

Discussion

We evaluated the associations between SNPs in candidate genes involved in the PI3-KAKT-mTOR signaling pathway and breast cancer DFS among a large cohort of patients with early stage breast cancer who received long-term follow-up. Evidence was strongest for a role of the *IGF1* rs1520220 SNP in reducing breast cancer DFS. Interestingly, the rs1520220 SNP in *IGF-1* has been shown to be associated with circulating serum levels of IGF-1 [24,25]. IGF-1 ligand binding with the IGF-1 receptor, leading to activation of downstream signaling pathways including the mitogen-activating protein kinase/extracellular signal-related kinase (ERK) and the PI3K pathway, contributes to IGF-1-mediated breast cancer cell proliferation and metastasis [26]. IGF-1 has been implicated in the survival of triple negative (ER, PR and Her2neu-negative) breast cancer cells [27] and with resistance to endocrine therapy in ER+ cell lines [28]. However, while high circulating serum levels of IGF-1 are a risk factor for developing breast cancer [29] there is conflicting epidemiological data on whether IGF-1 is

associated with breast cancer survival or recurrence [30,31]. Given the active development of inhibitory IGF-1 receptor monoclonal antibodies and small molecule receptor inhibitors as a novel cancer therapeutic strategy [32], identifying breast cancer patients with genetic susceptibility to increased signaling via the IGF-1 receptor may play an important role in personalizing treatment.

Obesity negatively affects breast cancer prognosis through disrupting inflammatory cytokines and adipokines, such as adiponectin and leptin that have anti-inflammatory and insulin-sensitizing properties [15, 16]. Adiponectin suppresses cell growth, induces apoptosis and angiogenesis [33]. In our study of breast cancer patients, the minor genotype C/C for SNP rs1063539 in *ADIPOQ* was associated with an improved breast cancer DFS. In a previous study, this genotype of SNP rs1063539 allele in *ADIPOQ* (which is in LD with *ADIPOQ* SNPrs3774262) was associated with lower body weight, waist circumference and BMI in healthy female controls [34]. No association was observed in a nested case-control study between SNPs in the *ADIPOQ* gene including the rs1063539 SNP and breast cancer risk among postmenopausal women [35], suggesting that any modification in energy balance or obesity associated with this genetic variant in the *ADIPOQ* gene may have a stronger influence on tumor survival than initiation.

The biological mechanisms through which tagging SNPs rs11585329 in *LEPR*, rs2519757 in *TSC1* and rs2677760 in *PIK3CA* (and the SNPs that they represent) influence breast cancer disease-free survival are not known and the possibility of a gene-gene or gene-environmental interaction cannot be ruled out. However, given the importance of the PI3K-AKT-mTOR pathway in breast cancer prognosis, evaluating the contribution of genetic variants of significant candidate genes to breast cancer DFS may have potential implications for identifying women at increased risk of recurrence or death who can be targeted for novel therapeutic interventions with mTOR inhibitors and IGFR1 monoclonal antibodies.

Our study had several limitations. First, we included only tagging SNPs therefore it is possible that a comprehensive screening approach for each candidate gene may have uncovered additional functionally relevant prognostic SNPs. Second, in this study set with long term follow-up, we were unable to measure serum levels of IGF-1, adiponectin or leptin and test for a correlation between the prognostic tag SNPs identified in *IGF-1*, *ADIPOQ* and *LEPROT*. In addition, we did not perform analyses according to tumor subtype although there was no evidence of an interaction of the significant SNPs with ER and PR status.

In summary, although we cannot rule out that the observed associations between SNPs in the PI3K-AKT-mTOR pathways and breast cancer DFS may have occurred by chance, the results of this study are biologically plausible given the functional significance of the tag SNPs rs1063539 in *ADIPOQ* and rs1520220 in *IGF-1* and the important role that obesity plays in breast cancer survival. Prior to attributing clinical significance to the study findings, replication of these results is warranted with evaluation of the relationship between these polymorphisms and activation of the PI3K-AKT-mTOR pathway in breast tumors.

Acknowledgments

This research was supported by the following grants--Susan Komen Career Catalyst Disparities Award (AMB), Komen Foundation Promise and SAC grant (GBM), Susan G. Komen for the Cure SAC110047 and SAB0800007 (MB), National Breast Cancer Foundation, and the National Cancer Institute at the NIH SPORE P50CA116199 (MB), National Institutes of Health R01 CA089608 (MB and PT), and National Institutes of Health K07 CA160753 (MP).

Abbreviations

PI3K	phosphatidylinositol-3 kinase
mTOR	mammalian target of rapamycin
ADIPOQ	adiponectin, C1Q and collagen domain containing
IGF1	Insulin-like growth factor 1
INS	Insulin
IRS1	Insulin receptor substrate 1
LEP	Leptin
LEPR	Leptin receptor
LEPROT	Leptin receptor overlapping transcript
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PIK3R5	Phosphoinositide-3-kinase, regulatory subunit 5
PTEN	Phosphatase and tensin homolog
TSC1	Tuberous sclerosis 1
TSC2	Tuberous sclerosis 2
AKT1	V-Akt murine thymoma viral oncogene homolog 1
SNP	single nucleotide polymorphism
DFS	disease-free survival
ESBCR	Early stage breast cancer registry
ER	estrogen receptor
PR	progesterone receptor
MAF	minor allele frequency
LD	linkage disequilibrium
HR	hazard ratio
CI	confidence interval

References

1. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov.* 2005; 4(12):988–1004. 2005. [PubMed: 16341064]
2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin.* 2014; 64(1):9–29. [PubMed: 24399786]
3. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev.* 2004; 18(16):1926–1945. [PubMed: 15314020]
4. Mamane Y, Petroulakis E, LeBacquer O, Sonenberg N. mTOR, translation initiation and cancer. *Oncogene.* 2006; 25(48):6416–6422. [PubMed: 17041626]
5. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. *Nat Rev Cancer.* 2004; 4(5):335–348. [PubMed: 15122205]
6. Dutcher JP. Mammalian target of rapamycin inhibition. *Clin Cancer Res.* 2004; 10(18 Pt 2):6382S–6387S. [PubMed: 15448035]
7. Berns K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell.* 2007; 12(4):395–402. [PubMed: 17936563]
8. Chandralapaty S, Sakr RA, Giri D, et al. Frequent mutational activation of the PI3K-AKT pathway in trastuzumab-resistant breast cancer. *Clin Cancer Res.* 2012; 18(24):6784–6791. [PubMed: 23092874]
9. Karlsson E, Perez-Tenorio G, Amin R, et al. The mTOR effectors 4EBP1 and S6K2 are frequently coexpressed, and associated with a poor prognosis and endocrine resistance in breast cancer: a retrospective study including patients from the randomised Stockholm tamoxifen trials. *Breast Cancer Res.* 2013; 15(5):R96. [PubMed: 24131622]
10. Kirkegaard T, Witton CJ, McGlynn LM, et al. AKT activation predicts outcome in breast cancer patients treated with tamoxifen. *J Pathol.* 2005; 207(2):139–146. [PubMed: 16088978]
11. Lu CH, Wyszomierski SL, Tseng LM, et al. Preclinical testing of clinically applicable strategies for overcoming trastuzumab resistance caused by PTEN deficiency. *Clin Cancer Res.* 2007; 13(19):5883–5888. [PubMed: 17908983]
12. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell.* 2004; 6(2):117–127. [PubMed: 15324695]
13. Razis E, Bobos M, Kotoula V, et al. Evaluation of the association of PIK3CA mutations and PTEN loss with efficacy of trastuzumab therapy in metastatic breast cancer. *Breast Cancer Res Treat.* 2011; 128(2):447–456. [PubMed: 21594665]
14. Tevaarwerk AJ, Gray RJ, Schneider BP, et al. Survival in patients with metastatic recurrent breast cancer after adjuvant chemotherapy: little evidence of improvement over the past 30 years. *Cancer.* 2013; 119(6):1140–1148. [PubMed: 23065954]
15. Chen M, Gu J, Delclos GL, et al. Genetic variations of the PI3K-AKT-mTOR pathway and clinical outcome in muscle invasive and metastatic bladder cancer patients. *Carcinogenesis.* 2010; 31(8):1387–1391. [PubMed: 20530239]
16. Sun CH, Chang YH, Pan CC. Activation of the PI3K/Akt/mTOR pathway correlates with tumour progression and reduced survival in patients with urothelial carcinoma of the urinary bladder. *Histopathology.* 2011; 58(7):1054–1063. [PubMed: 21707707]
17. Yoshioka A, Miyata H, Doki Y, et al. The activation of Akt during preoperative chemotherapy for esophageal cancer correlates with poor prognosis. *Oncol Rep.* 2008; 19(5):1099–1107. [PubMed: 18425364]
18. Brewster AM, Do KA, Thompson PA, et al. Relationship between epidemiologic risk factors and breast cancer recurrence. *J Clin Oncol.* 2007; 25(28):4438–4444. [PubMed: 17785707]
19. Carlson CS, Eberle MA, Rieder MJ, et al. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet.* 2004; 74(1):106–120. [PubMed: 14681826]
20. Thorisson GA, Smith AV, Krishnan L, Stein LD. The International HapMap Project Web site. *Genome Res.* 2005; 15(11):1592–1593. [PubMed: 16251469]

21. QIAamp® DNA Mini and Blood Mini Handbook. QIAGEN. 2010
22. Applied Biosystems® Arcturus® PicoPure® DNA Extraction Kit. 2010
23. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society*. 1995; 57:289–300.
24. Diorio C, Brisson J, Berube S, Pollak M. Genetic polymorphisms involved in insulin-like growth factor (IGF) pathway in relation to mammographic breast density and IGF levels. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(4):880–888. [PubMed: 18398029]
25. Gu F, Schumacher FR, Canzian F, et al. Eighteen insulin-like growth factor pathway genes, circulating levels of IGF-I and its binding protein, and risk of prostate and breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2010; 19(11):2877–2887. [PubMed: 20810604]
26. Siddle K. Signalling by insulin and IGF receptors: supporting acts and new players. *J Mol Endocrinol*. 2011; 47(1):R1–R10. [PubMed: 21498522]
27. Davison Z, de Blacquiére GE, Westley BR, May FE. Insulin-like growth factor-dependent proliferation and survival of triple-negative breast cancer cells: implications for therapy. *Neoplasia*. 2011; 13(6):504–515. [PubMed: 21677874]
28. Zhang Y, Moerkens M, Ramaiahgari S, et al. Elevated insulin-like growth factor 1 receptor signaling induces antiestrogen resistance through the MAPK/ERK and PI3K/Akt signaling routes. *Breast Cancer Res*. 2011; 13(3):R52. [PubMed: 21595894]
29. Key TJ, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *Lancet Oncol*. 2010; 11(6):530–542. [PubMed: 20472501]
30. Hartog H, Boezen HM, de Jong MM, et al. Prognostic value of insulin-like growth factor 1 and insulin-like growth factor binding protein 3 blood levels in breast cancer. *Breast*. 2013; 22(6):1155–1160. [PubMed: 23968866]
31. Pasanisi P, Venturelli E, Morelli D, et al. Serum insulin-like growth factor-I and platelet-derived growth factor as biomarkers of breast cancer prognosis. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(7):1719–1722. [PubMed: 18628423]
32. Mahadevan D, Sutton GR, Arteta-Bulos R, et al. Phase 1b study of safety, tolerability and efficacy of R1507, a monoclonal antibody to IGF-1R in combination with multiple standard oncology regimens in patients with advanced solid malignancies. *Cancer Chemother Pharmacol*. 2014; 73(3):467–473. [PubMed: 24390424]
33. Khan S, Shukla S, Sinha S, Meeran SM. Role of adipokines and cytokines in obesity-associated breast cancer: therapeutic targets. *Cytokine Growth Factor Rev*. 2013; 24(6):503–513. [PubMed: 24210902]
34. Chen X, Xiang YB, Long JR, et al. Genetic polymorphisms in obesity-related genes and endometrial cancer risk. *Cancer*. 2012; 118(13):3356–3364. [PubMed: 22038736]
35. Teras LR, Goodman M, Patel AV, et al. No association between polymorphisms in LEP, LEPR, ADIPOQ, ADIPOR1, or ADIPOR2 and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2009; 18(9):2553–2557. [PubMed: 19723917]

Fig. 1. Kaplan-Meier graph comparing breast cancer disease-free survival for *IGF1* rs1520220 homozygous wild-type genotype (WW) with that for heterozygous and homozygous variant genotypes (WM/MM)

Table 1

Univariate associations of demographic and clinical characteristics with breast cancer disease-free survival (DFS)

Covariate	No Event		Event	
	n(%)		n(%)	HR (95% CI) ^a
Years of survival, mean (range)	10.5(0.2,22.2)		4.7(.2,21.5)	
Age at diagnosis, years				
<50	288(38.1)		146(55.5)	Reference
50	468(61.9)		117(44.5)	0.58(0.46–0.74)
Race				
White	550(72.8)		186(70.7)	Reference
African American	97(12.8)		38(14.4)	1.18(0.83–1.67)
Hispanic	86(11.4)		29(11)	1.12(0.76–1.65)
Unknown	23(3)		10(3.8)	-
Stage				
I	251(33.2)		51(19.4)	Reference
II	505(66.8)		212(80.6)	1.87(1.38–2.55)
Tumor size (cm)				
0	35(4.6)		16(6.1)	Reference
>0–2	462(61.1)		115(43.7)	0.61(0.36–1.02)
>2	252(33.3)		127(48.3)	1.07(0.63–1.79)
Unknown	7(0.9%)		5(1.9)	-
Lymph node status				
Negative	459(60.7)		132(50.2)	Reference
Positive	297(39.3)		131(49.8)	1.41(1.10–1.79)
Nuclear grade				
1	251(33.2)		94(35.7)	Reference
2	405(53.6)		129(49)	0.86(0.66–1.12)
3	72(9.5)		25(9.5)	0.98(0.63–1.53)
Unknown	28(3.7)		15(5.7)	-
ER/PR				
Negative	162(21.4)		72(27.4)	Reference
Positive	562(74.3)		174(66.2)	0.72(0.55–0.95)
Unknown	32(4.2)		17(6.5)	-
BMI (kg/m²)				
<25	314(41.5)		111(42.2)	Reference
25–<30	212(28)		82(31.2)	1.09(0.82–1.45)
30	213(28.2)		62(23.6)	0.91(0.66–1.24)
Unknown	17(2.2)		8(3)	-
Treatment (Hormone and Chemotherapy)				
Neither	162(21.4)		66(25.1)	Reference

Covariate	No Event	Event	
	n(%)	n(%)	HR (95% CI) ^a
Both	386(51.1)	150(57)	0.97(0.73–1.30)
Hormone only	204(27)	47(17.9)	0.63(0.43–0.92)
Unknown	4(0.5)	-	-

^aHR – hazard ratio; CI – confidence interval

Table 2
SNPs significantly associated with breast cancer disease-free survival in multivariable Cox regression analysis

SNP	Gene	Location	MAF ^a	Model	p	HR ^a	95% CI ^a
rs1063539	ADIPOQ	3UTR	0.11	Dominant	0.02	0.40	0.19–0.86
rs11585329	LEPR	Intron	0.13	Recessive	0.04	0.72	0.53–0.98
rs1520220	IGF1	Intron	0.20	Recessive	0.001	1.56	1.21–2.00
rs2519757	TSC1	Intron	0.05	Dominant	0.03	0.29	0.09–0.91
rs2677760	PIK3CA	Intron	0.45	Recessive	0.02	1.43	1.07–1.92

^aMAF, minor allele frequency; HR, hazard ratio; CI, confidence interval.

Table 3
Association of individual SNPs with breast cancer disease-free survival stratified by body mass index (BMI)^a

BMI kg/m ²	SNP	Gene	Model	P	HR ^b	95% CI ^b
<25 2	rs1063539	ADIPOQ	Dominant	0.03	0.33	0.12
				0.44	0.63	0.20 2.01
<25 25	rs11585329	LEPR	Recessive	0.27	0.78	0.51
				0.06	0.65	0.42 1.02
<25 25	rs1520220	IGF1	Recessive	0.06	1.45	0.99
				0.01	1.61	1.15 2.25
<25 25	rs2519757	TSC1	Dominant	0.46	0.48	0.07
				0.003	0.11	0.03 0.47
<25 25	rs2677760	PIK3CA	Recessive	0.59	1.13	0.72
				0.04	1.50	1.01 2.21

^a Adjusted for race/ethnicity, age at diagnosis, cancer stage, and treatment

^b HR, hazard ratio; CI, confidence interval