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Germline genome-wide association studies in women receiving neoadjuvant chemotherapy with or without bevacizumab

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Abstract

Neoadjuvant chemotherapy (NAC) for breast cancer is widely employed and we performed genome-wide association studies (GWAS) to determine if germline genetic variability was associated with benefit in terms of pathological complete response (pCR), disease-free survival

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(DFS), and overall survival (OS) in patients entered on the NSABP B-40 NAC trial where patients were randomized to receive, or not, bevacizumab in addition to chemotherapy. Patient DNA samples were genotyped with the Illumina OmniExpress BeadChip. Replication was attempted with genotyping data from 1398 HER2-negative patients entered on the GeparQuinto NAC study in which patients were also randomized to receive, or not, bevacizumab in addition to chemotherapy. 920 women from B-40 were analyzed and 237 patients achieved a pCR. GWAS with three phenotypes (pCR, DFS, OS) revealed no SNPs that were genome-wide significant (i.e., p≤5E-08) signals; p-values for top SNPs were 2.04E-07, 5.61E-08, and 5.63E-08, respectively, and these SNPs were not significant in the GeparQuinto data. An *ad hoc* GWAS was performed in the patients randomized to bevacizumab (457 patients with 128 pCR) that showed signals on chromosome 6, located within a gene, *CDKAL1,* that approached, but did not reach, genome-wide significance (top SNP rs7453577, p=2.97E-07). However, this finding was significant when tested in the GeparQuinto dataset (p=0.04). In conclusion, we identified no SNPs significantly associated with NAC. The observation, in a hypothesis-generating GWAS, of a SNP in *CDKAL1* associated with pCR in the bevacizumab arm of both B-40 and GeparQuinto requires further validation and study.

Keywords

neoadjuvant chemotherapy; bevacizumab; breast cancer; pharmacogenomics

Introduction

There is increasing use of neoadjuvant chemotherapy (NAC) in the management of early-stage breast cancer(1). Based on the importance of angiogenesis in breast cancer progression(2) and promising results from early studies of bevacizumab, a monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A), in metastatic breast cancer(3), multiple clinical trials in the neoadjuvant setting were conducted. Four of these trials have reported results, including NSBAP B-40(4, 5), GeparQuinto(6, 7), CALGB 40603(8), and the ARTemis trial(9).

Achievement of a pathological complete response (pCR), with complete eradication of invasive breast cancer in the breast and nodes, has been associated with improved survival with the greatest prognostic value in aggressive tumor subtypes(10). We hypothesized that there are genes related to the achievement of a pCR in women treated with NAC with or without bevacizumab, and that we would be able to identify germ-line genetic variation measured as single nucleotide polymorphisms (SNPs) associated with pCR with a genome-wide association study (GWAS) utilizing B-40(4) and attempting replication with HER2-negative patients entered on GeparQuinto(6).

Methods

Source of Patients

All patients with HER2-negative early breast cancer entered on NSABP B-40 (schema: Supplementary Figure 1) with a blood sample for DNA extraction and consent for genetic testing were eligible following Mayo IRB review.

Definition of Phenotype

The pCR definition was the complete eradication of all invasive breast cancer in both the breast and regional nodes.

Study Design

Anonymized samples were sent to Mayo Clinic for DNA extraction, were plated, and sent to the RIKEN Center for Integrative Medical Science for genotyping. The clinical and genotyping data were then analyzed at Mayo Clinic. The primary objective was to identify genetic variation measured as SNPs associated with pCR with a GWAS. Secondary objectives were to explore the association of SNPs with outcomes, i.e., disease-free survival (DFS) and overall survival (OS). An *ad hoc* exploratory GWAS was performed with the phenotype of pCR in patients randomized to bevacizumab.

Genotyping, quality control, and imputation

Genotyping was performed with the Illumina HumanOmniExpressExome BeadChips. Details regarding genotyping, quality control, and imputation are given the Supplementary Material.

The data from this GWAS have been deposited in the Data Base of Genotypes and Phenotypes (dbGaP). The dbGaP Study Accession Number is phs001365.v1.p1 and the URL is https://www.ncbi.nlm.nih.gov/projects/gapprev/gap/cgi-bin/preview1.cgi?GAP_phs_code=DlBPhGnsRxXYbJZW.

Statistical analysis

The primary analyses were based on logistic regression with SNP genotypes analyzed as log-additive effects on the chance of a pCR. The primary covariates that were adjusted for include treatment arm and any other clinical factors found to be associated with pCR (at p-value < 0.10). To control for potential population stratification, we used the program STRUCTURE and HapMap racial groups to determine additional covariates. We utilized EigenStrat to determine the eigen values for the SNP correlation matrix that statistically differed from zero(11, 12). To evaluate the association of SNPs with DFS and OS, we used the Cox proportional hazards model, including covariates to adjust for patient heterogeneity.

Replication

Replication of top SNPs from the four GWAS (pCR, DFS, OS, and pCR in bevacizumab patients) was attempted utilizing genotyping data from patients with HER2-negative early breast cancer entered on GeparQuinto (schema: Supplementary Figure 2, Supplementary

Table). Details regarding genotyping, quality control, imputation, and sample cohorts for individual GWAS of GeparQuinto are given in the Supplementary Material.

Results

Patients studied

The participant flow diagram (Supplementary Figure 3) shows the B-40 patients included and excluded from the four GWAS with the phenotypes of 1) pCR, 2) DFS, 3) OS, and 4) pCR in bevacizumab-treated patients only. Supplementary Figure 4 shows the GeparQuinto patients included in the replication studies.

GWAS with phenotype of PCR

Table 1 shows the clinical summary of the 914 patients in the primary analysis. The analysis was adjusted for treatment (bevacizumab, no bevacizumab), race, completion of neoadjuvant chemotherapy, and tumor grade in addition to being stratified for categorical-age, ER/PR status, tumor size and nodal stage. The distribution of p-values is shown in the Manhattan plot (Figure 1A) and locus zoom (Supplementary Figure 5A) and revealed the top SNP (rs34843881, imputed) on chromosome 13 to have a p-value of 2.04E-07, which did not reach genome-wide statistical significance (Table 2). The Quantile-Quantile (QQ) plot for the conditional logistic regression results is shown in Supplementary Figure 6A. The top SNP was examined in GeparQuinto and showed p=0.73 (Table 2), indicating this was not an important signal.

GWAS with phenotype of disease-free survival

The GWAS with the phenotype of DFS was performed with 890 patients since 24 of the 914 patients were removed due to missing outcome data and/or tumor grade. A DFS event occurred in 219 (24.6%) of the patients. Stepwise analysis showed clinical variables such as treatment, tumor grade, and completion of neoadjuvant chemotherapy were associated with DFS and these variables were controlled for in the analysis, in addition to race. The Manhattan plot (Figure 1B) and locus zoom (Supplementary Figure 5B) revealed the top SNP (rs78269823, imputed) on chromosome 14 to have a p-value of 5.61E-08, which approached but did not reach genome-wide significance. The QQ plot is shown in Supplementary Figure 6B. The top SNP was examined in GeparQuinto and showed p=0.76 (Table 2), indicating this was not an important signal.

GWAS with phenotype of overall survival

The GWAS with the phenotype of OS was performed with 891 patients of whom 144(16.1%) had died. The model was controlled by the same variables as for DFS. The Manhattan plot (Figure 1C) and locus zoom (Supplementary Figure 5C) revealed the top SNP (rs56330643, imputed) on chromosome 14 to have a p-value of 5.63E-08, which approached but did not reach genome-wide significance. The QQ-plot is shown in Supplementary Figure 6C). The top SNP was examined in GeparQuinto and showed p=0.50 (Table 2), indicating this was not an important signal.

Exploratory GWAS in patients who received bevacizumab

The GWAS with the phenotype of pCR was performed with 447 patients who had received bevacizumab of whom 147 (32.8%) had achieved a pCR. The model was controlled for the stratification variables noted above, race, and tumor grade. The Manhattan plot (Figure 1D) and locus zoom (Supplementary Figure 5D) revealed the top SNP, (rs7453577, imputed) to have a p-value of 2.97E-07. The QQ plot is shown in Supplementary Figure 6D. The top SNP was examined in GeparQuinto and showed p=0.04 (Table 2), which achieved statistical significance.

Given the findings from GeparQuinto, we examined this area more closely. In the B-40 GWAS, there were a total of 17 SNPs in addition to the top SNP in a gene, *CDKAL1* (CDK5 Regulatory Subunit Associated Protein 1 Like 1), with p-values ranging from 3.11E-07 to 8.63E-07. Included in these SNPs was a single genotyped SNP, rs1004172, with p=3.73E-07. The MAFs of these SNPs were 0.19–0.22.

Discussion

The primary objective of this study was to identify any association between germ-line genetic variation and pCR in women receiving NAC in B-40. The top SNP (rs34843881) did not achieve genome-wide significance (p=2.04 E-07). When this SNP was examined in GeparQuinto database there was no evidence of any association (p=0.73).

Secondary objectives were to identify any association between germ-line genetic variation and the outcomes of DFS and OS in women receiving NAC. The top SNPs from B-40 almost reached genome-wide significance, p-value accepted to be 5E-08, i.e., 5.61E-08 and 5.63E-08, respectively. However, when examined in GeparQuinto, the p-values were not replicated, being p=0.76 and p=0.50, respectively.

Because those studies reported a significantly higher pCR rate in the patients randomized to bevacizumab, we performed an exploratory GWAS only in the patients who received bevacizumab. The top SNP (rs7453577) had a p-value of 2.97E-07 in B40 and when examined in GeparQuinto was significant (p=0.04). Given that this was an *ad hoc* analysis, it must be considered hypothesis generating. The top SNP was located in *CDKAL1*, which encodes a protein that is a member of the methylthiotransferase family. The function of this gene is not known, but prior GWAS have linked SNPs in an intron of *CDKAL1* with susceptibility to type II diabetes(13).

Ideally, a replication study should be identical to a discovery study. Whereas B-40 and GeparQuinto have substantial similarities, they also have differences. The anthracycline utilized was different being doxorubicin in B-40 and epirubicin in GeparQuinto. The sequencing of the anthracycline and taxane was opposite and the duration of bevacizumab therapy was different in the two studies. Patients were taken off study if a response was not seen after the 4 cycles of the anthracycline plus cyclophosphamide in the GeparQuinto study. Also, in B-40, bevacizumab had a significantly higher pCR rate in ER-positive patients whereas in GeparQuinto the bevacizumab had a significantly higher pCR rate in

ER-negative patients. These differences demonstrate that in pharmacogenomics, the identification of an ideal replication study can be difficult.

Conclusions

We performed GWAS in a major NAC study and did not find a significant association between germ-line genetic variation and pCR, DFS, or OS. The observation of a SNP in *CDKAL1* associated with pCR in the bevacizumab arm of both B40 and GeparQuinto requires further validation and study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, et al. Tailoring therapies-improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol. 2015; 26:1533–46. [PubMed: 25939896]
2. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. N Engl J Med. 1991; 324:1–8.
3. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med. 2007; 357:2666–76. [PubMed: 18160686]
4. Bear HD, Tang G, Rastogi P, Geyer CE Jr, Robidoux A, Atkins JN, et al. Bevacizumab added to neoadjuvant chemotherapy for breast cancer. N Engl J Med. 2012; 366:310–20. [PubMed: 22276821]
5. Bear HD, Tang G, Rastogi P, Geyer CE Jr, Liu Q, Robidoux A, et al. Neoadjuvant plus adjuvant bevacizumab in early breast cancer (NSABP B-40 [NRG Oncology]): secondary outcomes of a phase 3, randomised controlled trial. Lancet Oncol. 2015; 16:1037–48. [PubMed: 26272770]
6. von Minckwitz G, Eidtmann H, Rezai M, Fasching PA, Tesch H, Eggemann H, et al. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. N Engl J Med. 2012; 366:299–309. [PubMed: 22276820]
7. von Minckwitz G, Loibl S, Untch M, Eidtmann H, Rezai M, Fasching PA, et al. Survival after neoadjuvant chemotherapy with or without bevacizumab or everolimus for HER2-negative primary breast cancer (GBG 44-GeparQuinto)dagger. Annals of oncology : official journal of the European Society for Medical Oncology. 2014; 25:2363–72. [PubMed: 25223482]
8. Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol. 2015; 33:13–21. [PubMed: 25092775]

9. Earl HM, Hiller L, Dunn JA, Blenkinsop C, Grybowicz L, Vallier AL, et al. Efficacy of neoadjuvant bevacizumab added to docetaxel followed by fluorouracil, epirubicin, and cyclophosphamide, for women with HER2-negative early breast cancer (ARTemis): an open-label, randomised, phase 3 trial. Lancet Oncol. 2015; 16:656–66. [PubMed: 25975632]
10. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014; 384:164–72. [PubMed: 24529560]
11. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. PLoS Genet. 2006; 2:e190. [PubMed: 17194218]
12. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–9. [PubMed: 16862161]
13. Palmer CJ, Bruckner RJ, Paulo JA, Kazak L, Long JZ, Mina AI, et al. Cdkal1, a type 2 diabetes susceptibility gene, regulates mitochondrial function in adipose tissue. Mol Metab. 2017; 6:1212–25. [PubMed: 29031721]

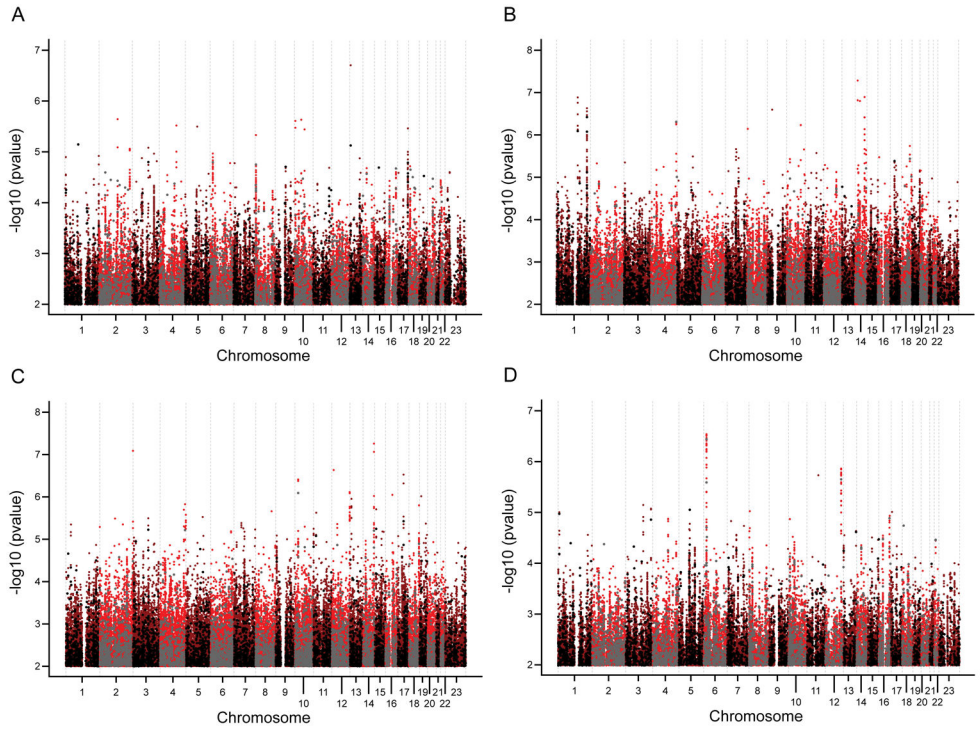

Figure 1.

Manhattan plots of p-values for conditional logistic regression analysis of the NSABP B-40 trial for A) pathologic complete response (pCR), B) disease-free survival, C) overall survival, and D) pCR in bevacizumab-treated patients only.

Table 1

NSABP B-40 Clinical Summary

	No Bevacizumab (N=459)	Bevacizumab (N=455)	Total (N=914)
Age at Randomization			
Median	49.0	48.0	49.0
Q1, Q3	41.0, 56.0	42.0, 56.0	41.0, 56.0
Range	(25.0–70.0)	(24.0–71.0)	(24.0–71.0)
pCR Breast and Nodes			
No	350 (76.3%)	328 (72.1%)	678 (74.2%)
Yes	109 (23.7%)	127 (27.9%)	236 (25.8%)
Gemcitabine			
No	314 (68.4%)	305 (67.0%)	619 (67.7%)
Yes	145 (31.6%)	150 (33.0%)	295 (32.3%)
Capecitabine			
No	303 (66.0%)	301 (66.2%)	604 (66.1%)
Yes	156 (34.0%)	154 (33.8%)	310 (33.9%)
Bevacizumab			
No	459 (100.0%)	0 (0.0%)	459 (50.2%)
Yes	0 (0.0%)	455 (100.0%)	455 (49.8%)
Clinical Tumor Status			
2–4cm	212 (46.2%)	202 (44.4%)	414 (45.3%)
>4cm	247 (53.8%)	253 (55.6%)	500 (54.7%)
Clinical Nodal Status			
Negative	243 (52.9%)	251 (55.2%)	494 (54.0%)
Positive	216 (47.1%)	204 (44.8%)	420 (46.0%)
Hormone Receptor Status			
Negative	197 (42.9%)	193 (42.4%)	390 (42.7%)
Positive	262 (57.1%)	262 (57.6%)	524 (57.3%)
Estrogen Receptor/Progesterone Receptor			
Negative/Negative	197 (43.2%)	193 (42.4%)	390 (42.8%)
Negative/Positive	8 (1.8%)	9 (2.0%)	17 (1.9%)
Positive/Negative	40 (8.8%)	47 (10.3%)	87 (9.5%)
Positive/Positive	211 (46.3%)	206 (45.3%)	417 (45.8%)
Positive/unknown	3 (0.6%)	0	3 (0.3%)
Histologic Tumor Grade			
Well	33 (7.2%)	30 (6.6%)	63 (6.9%)
Moderate	149 (32.5%)	160 (35.2%)	309 (33.8%)
Poor	269 (58.6%)	261 (57.4%)	530 (58.0%)
Unknown	8 (1.7%)	4 (0.9%)	12 (1.3%)
Breast			

	No Bevacizumab (N=459)	Bevacizumab (N=455)	Total (N=914)
Left	227 (49.5%)	226 (49.7%)	453 (49.6%)
Right	232 (50.5%)	229 (50.3%)	461 (50.4%)
Completion of Neoadjuvant Treatment Protocol			
Yes	372 (81.0%)	353 (77.6%)	725 (79.3%)
No	87 (19.0%)	102 (22.4%)	189 (20.7%)
structure_race			
AA	69 (15.0%)	51 (11.2%)	120 (13.1%)
CA	352 (76.7%)	377 (82.9%)	729 (79.8%)
HC	13 (2.8%)	4 (0.9%)	17 (1.9%)
UN	25 (5.4%)	23 (5.1%)	48 (5.3%)

Table 2

Top SNP from the GWAS for pCR, DFS, OS, and bevacizumab-treated patients with replication utilizing GeparQuinto

						B40						GeparQuinto					
	rsID	**CHR**	**POS**	**CA**	**MA**	**N**	**MAF**	**OR**	**lci**	**uci**	**pvalue**	**N**	**MAF**	**OR**	**lci**	**uci**	**Pvalue**
pCR	rs34843881	13	25971560	C	T	914	0.096	2.899	1.940	4.331	2.04E−07	1398	0.095	0.937	0.650	1.352	0.73
DFS	rs78269823	14	39264849	C	T	890	0.067	0.197	0.093	0.416	5.61E−08	1398	0.117	0.952	0.688	1.316	0.76
OS	rs56330643	14	100388910	A	G	891	0.297	2.050	1.588	2.645	5.63E−08	1398	0.357	0.926	0.738	1.161	0.50
Bev, pCR	rs7453577	6	20987675	G	A	447	0.203	2.734	1.861	4.016	2.97E−07	733	0.209	1.498	1.029	2.182	0.04

rsID: reference sequence ID,