Genetic variants in VEGF pathway genes in neoadjuvant breast cancer patients receiving bevacizumab: results from the randomized phase 3 GeparQuinto study

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Running title:

VEGF SNPs and bevacizumab response in the GeparQuinto trial

Novelty & Impact Statements:

In this pre-planned biomarker study of the neoadjuvant GeparQuinto trial, germline genetic variants in VEGF pathway genes were associated with bevacizumab treatment outcome, as assessed by pathological complete response. In particular, four variants in *VEGFA* and one variant in *FLT1* were associated with treatment outcome. The effect of some variants seemed more pronounced in patients with triple-negative tumors.

Abstract

Studies assessing the effect of bevacizumab (BEV) on breast cancer outcome have shown different effects on progression-free and overall survival, suggesting that a subgroup of patients may benefit from this treatment. Unfortunately, no biomarkers exist to identify these patients. Here, we investigate whether single nucleotide polymorphisms (SNPs) in VEGF pathway genes correlate with pathological complete response (pCR) in the neoadjuvant GeparQuinto trial.

HER2-negative patients were randomized into treatment arms receiving either BEV combined with standard chemotherapy or chemotherapy alone. In a pre-planned biomarker study, DNA was collected from 729 and 724 patients, respectively from both treatment arms, and genotyped for 125 SNPs. Logistic regression assessed interaction between individual SNPs and both treatment arms to predict pCR.

Five SNPs may be associated with a better response to BEV, but none of them remained significant after correction for multiple testing. The two SNPs most strongly associated, rs833058 and rs699947, were located upstream of the *VEGFA* promoter. Odds ratios for the homozygous common, heterozygous and homozygous rare rs833058 genotypes were 2.36 (95%CI, 1.49–3.75), 1.20 (95%CI, 0.88–1.64) and 0.61 (95%CI, 0.34–1.12). Notably, some SNPs in *VEGFA* exhibited a more pronounced effect in the triple-negative subgroup.

Several SNPs in *VEGFA* may be associated with improved pCR when receiving BEV in the neoadjuvant setting. Although none of the observed effects survived correction for multiple testing, our observations are consistent with previous studies on BEV efficacy in breast cancer. Further research is warranted to clarify the predictive value of these markers.

Key words: breast cancer, VEGF, anti-angiogenesis, bevacizumab, genetic variant, prognosis, biomarker, SNP

Introduction

Bevacizumab (BEV), a monoclonal antibody vascular endothelial growth factor-A (VEGFA), was the first anti-angiogenic drug to be approved for the treatment of breast cancer. Although it slows the progression of metastatic breast cancer when combined with chemotherapy,[1-4] the Food and Drug Administration withdrew approval for this indication in 2011 due to *(i)* a lack of evidence for a consistent overall survival (OS) benefit, *(ii)* the frequent occurrence of BEV-related toxicities, and *(iii)* the fact that there are no validated biomarkers to predict which patients really benefit from the therapy. [5] Although the therapeutic effect of BEV indeed appears to be modest in the metastatic breast cancer setting, the European Medicines Agency maintained its approval status. Similarly, recent neoadjuvant studies have shown a modest but significant increase in the pathological complete response (pCR) in HER2-negative breast cancer patients.[6, 7] In adjuvant studies, however, no benefit with regard to the disease-free survival has been demonstrated for patients with triple-negative (TN) or HER2-positive breast cancer (BC).[8, 9]

Markers capable of predicting the response to anti-angiogenic treatment or the occurrence of side effects are thus urgently needed, as this might improve patient selection for these patients. With regard to breast cancer subtypes, the GeparQuinto trial has shown that patients with triple-negative breast cancer (TNBC) appear to benefit more from BEV treatment than non-TNBC patients.[6] However, this effect was not seen in the NSABP-B40 study.[7] Several other biomarkers predictive of BEV treatment were identified in retrospective studies, including high levels of tumor-associated VEGF or high circulating levels of plasma VEGF.[8, 10-12] In another study, gene amplifications involving *VEGFA* were associated with a poor outcome specifically in the subgroups of triple-negative and HER2-positive breast cancers.[13]

In this study, we hypothesize that germline genetic variants underlie the response to the anti-angiogenic effects of BEV. Angiogenesis is a process that affects tumor microenvironment and by affecting vessel regression and permeability only indirectly affects the tumor itself. Since endothelial cells, unlike tumor cells, are not affected by somatic alterations, germline genetic variants could indeed explain why some patients respond and others do not respond to anti-angiogenic therapy. Various potential predictive genetic markers have been identified in clinical trials evaluating BEV in several tumor types,[13-19] but these analyses — generally using single-trial datasets — are limited by the number of available samples, heterogeneity in the selection of single nucleotide polymorphisms (SNPs), and a lack of understanding of the functional consequences of these genetic markers. An analysis of two

randomized phase III trials with BEV in metastatic pancreatic adenocarcinoma (AViTA) and metastatic renal-cell carcinoma (AVOREN) has shown a significant correlation between rs9582036, a SNP in *FLT1*, and both PFS and OS in the BEV group only.[16] Trials in metastatic colorectal cancer have also reported significant associations with SNPs and BEV efficacy,[14, 15] although these results often lack confirmation.[17] With respect to breast cancer, a retrospective analysis of the phase 3 E2100 trial involving BEV revealed an association between several VEGF SNPs and either OS (rs699947 and rs1570360) or severe hypertension (rs2010963).[19] In another single-arm trial (MO19391) including 137 women, it was confirmed that rs2010963 in *VEGFA* is related to BEV-associated toxicity, whereas rs3025039 in *VEGFA* tended to be associated with time-to-progression. A recent meta-analysis of 6 different cancer types, including breast cancer, has also shown that several VEGF SNPs were predictive of PFS and also functionally relevant because they affected VEGFA gene expression, although observed effects were generally quite modest.[20]

The primary aim of this study was to assess whether SNPs in VEGF pathway genes are associated with efficacy of bevacizumab in all patients participating to the neoadjuvant breast cancer study, GeparQuinto, and as a secondary aim whether specific breast cancer subgroups, as defined by hormone receptor status, were more strongly associated to specific genetic variants.

Material and methods

Study population

The GeparQuinto phase III trial (NCT00567554) recruited patients with HER2-positive breast cancer for an anti-HER2 therapy–related study aim,[21] and HER2-negative patients with the aim of testing the efficacy of BEV.[6, 22] In the present study only HER2-negative patients from the GeparQuinto trial were included. The patients were treated with anthracycline and taxane neoadjuvant chemotherapy and were randomly assigned to either simultaneous treatment with bevacizumab or no additional therapy. In total, 1984 patients were randomly assigned in the main study. Germline DNA was available for 1503 of these patients. Some patients were excluded according to the following hierarchical order: patients without final surgery (n = 5), patients with >20% missing genotype, patient or tumor data (n=45). This resulted in a final sample size of 1453 patients. Of these patients, 729 were treated with bevacizumab and 724 were assigned to the control arm. The pharmacogenetic analysis was a preplanned biomarker substudy. All of the patients provided written informed consent for the pharmacogenetic analysis, and the relevant ethics committees at the participating study sites approved the study protocol.

Treatment and assessment of clinical data

The assessment of the clinical data has been described in detail elsewhere.[6] Briefly, patients included in this study were HER2-negative and scheduled to receive epirubicin and cyclophosphamide on day 1 and every 3 weeks for a total of four cycles, followed by docetaxel as well on day 1 and every 3 weeks for a total of four cycles. Patients were randomly assigned to receive either bevacizumab plus chemotherapy or chemotherapy alone. If there was no sonographical response after four cycles, patients were taken off treatment and continued with a different treatment.[23] For the present study, however, the patients were analyzed according to the planned therapy intention if they received at least one cycle of chemotherapy or at least one cycle of BEV. All clinical and histopathological data were assessed by the local pathologists, including tumor stage, HER2 status, estrogen receptor/progesterone receptor status, and grading. Pathological reports were assessed and reviewed centrally for the primary study aim. For this analysis, pathological response was defined as pathological stage pT0 and pN0 after therapy.

DNA extraction and genotyping

Whole blood samples were collected in citrate-phosphate-dextrose-adenine (CPDA) tubes (Sarstedt AG, Nümbrecht, Germany) from patients who had consented to participate in the biomarker substudy. Germline DNA was extracted using the automated magnetic bead–based chemagic MSM I technique (PerkinElmer chemagen, Baesweiler, Germany) in accordance with the manufacturer's instructions. Genotyping was done at the Vesalius Research Center, Leuven, Belgium using MassARRAY iPLEX Gold (Sequenom, San Diego, California, USA), as reported previously.[16]

Selection of SNPs

SNPs in the following 15 genes involved in the VEGF-A pathway were selected: *VEGFA,* the *VEGFA* homologs (placental growth factor [*PGF*], *VEGFB, VEGFC,* and *VEGFD* [also known as c-fos-induced growth factor or *FIGF*]), VEGF receptor-1 (*VEGFR1* or *FLT1*), VEGF receptor-2 (*VEGFR2* or *KDR*), VEGF receptor-3 (*VEGFR3* or *FLT4*) and the neuropilin-1 coreceptor (*NRP1*), regulators of hypoxia (hypoxia-inducible factor-1α [*HIF1A*], HIF-2α [*EPAS1*], factor inhibiting HIF-1A [*HIF1AN* or *FIH1*], von Hippel–Lindau tumor suppressor [*VHL*]), and the oxygen sensors (prolyl hydroxylase domain-containing proteins 1 [*EGLN2*], 2 [*EGLN1*], and 3 [*EGLN3*]). A detailed description of the way in which SNPs were selected in these genes has been published previously.[16] Briefly, genomic sequences 5 kb upstream of the translation start site and downstream of the 3' polyadenylation site in each gene were used to select SNPs from the HapMap database (phase 2 public release, no. 22). Common SNPs with a minor allele frequency ≥ 0.1 and pairwise correlation coefficients

(r^2) ≤ 0.8 were selected using the SNP Tagger approach in the Haploview software package.[24, 25] A total of 170 tagging SNPs in VEGF pathway genes, as well as 16 SNPs previously associated with bevacizumab efficacy or with circulating VEGF levels, were selected. Of these, 170 were successfully designed and genotyped (**Supplementary Table 1**). Quality control further removed 30 SNPs with a call rate of <95%. After exclusion of SNPs with a minor allele frequency < 0.1 in the study, a final set of 125 SNPs was considered for statistical analysis (**Supplementary Table 1**).

Statistical analyses

Logistic regression models were used as described below to investigate the association between the treatment arm and several SNPs relative to pCR. Patients with missing pCR information were excluded. Missing information about patient and tumor characteristics was imputed using single "best guesses" (median value of continuous predictors, the most common value of categorical or ordinal categorical predictors) based on non-missing data across all subjects. Missing genotype values were not imputed, but remained missing. Continuous predictors were used as natural cubic spline functions to describe nonlinear effects as recently described.[26]

For each SNP, a logistic regression model was fitted with the outcome pCR and the predictors SNP (ordinal; 0, 1, 2 minor alleles), treatment arm (bevacizumab versus standard treatment), SNP by treatment arm interaction, as well as the established predictors age (continuous), body mass index (continuous), tumor stage (ordinal), lymph-node status (categorical; pN0 versus pN+), grading (ordinal; G1, G2, G3), histology (categorical; ductal, lobular, others), and hormone receptor status (categorical; triple-negative versus not triple-negative). The SNP by treatment arm interaction was included to examine whether the differences between the treatment arms depended on genetic variants. The interaction P values were calculated and then corrected using the Bonferroni–Holm method. Genotype-specific odds ratios (ORs) for the treatment arm, adjusted for the established predictors from above, were estimated for ten SNPs with the smallest uncorrected P values using the interaction models.

The association between hormone receptor, genotypes, and treatment arm with regard to pCR was the secondary study aim. Therefore, for each of the ten SNPs with small interaction P values from above, the interaction model was extended by including the three-way interaction SNP by treatment arm by hormone receptor and the corresponding two-way interactions. The two models were compared using the Akaike information criterion (AIC). A better AIC value for the latter model suggests that the association of treatment and genotype differs between patients with triple-negative tumors and patients without triple-negative tumors. In this case, hormone

receptor and genotype-specific ORs for the treatment arm were estimated using the extended regression model.

The goodness of fit of the logistic regression models was tested using the Hosmer-Lemeshow χ^2 test, where a large *P* value indicates good calibration.

All of the tests were two-sided, and a *P* value of < 0.05 was regarded as statistically significant. Calculations were carried out using the R system for statistical computing (version 3.0.1; R Development Core Team, Vienna, Austria, 2013).

Results

Patient characteristics

A total of 1453 patients were included in the analysis. Complete data for patient and tumor characteristics were available for 95.7% of the patients. Patients and tumor characteristics are shown in **Table 1**. pCR was correlated with all previously described predictors in the expected direction. Patients with a higher tumor stage were less likely to achieve a pCR, and patients with a negative hormone receptor status or a higher grading were more likely to achieve a pCR (**Table 1**). The patient characteristics were similar to those in the main study. There were no differences in the pCR rate between patients who took part in this study and those who did not (data not shown).

Genotype results

Genotyping results, SNP location, and *P* values for the Hardy–Weinberg equilibrium analysis are presented in **Supplementary Table 1**.

The association between treatment and genotype with regard to pCR was analyzed using logistic regression models as described above. Ten SNPs with the smallest *P* values for SNP by treatment interaction are shown in **Table 2**. None of the SNPs were significant after correction for multiple testing. In other words, it was not shown that a genotype influenced the difference between the treatment arms.

Five of these 10 SNPs are located on chromosome 6 around *VEGFA,* and the top hit is located upstream from the promoter of *VEGFA* (**Fig. 1**). Three of the SNPs were located on chromosome 2 around *EPAS1*. Two further SNPs were related to *FLT1* and *EGLN3*.

Patients with homozygous common genotypes in *VEGFA* or *EPAS1* appeared to benefit from BEV treatment, whereas patients with at least one minor allele did not (**Table 2**). For example, in relation to rs833058 (*VEGFA*), patients with the common

genotype had a greater benefit from BEV treatment, with an OR of 2.36 (95% CI, 1.49 to 3.75), whereas patients with a heterozygous or homozygous rare genotype did not benefit (OR for heterozygous: 1.21; 95% CI, 0.89 to 1.65; OR for homozygous rare: 0.62, 95% CI, 0.34 to 1.13).

Next, the predictive association between treatment and genotype in these SNPs was assessed in the subgroups of hormone receptor–positive and hormone receptor–negative patients. Since this study was performed on HER2-negative patients, all hormone receptor–negative patients represent TNBC patients. The treatment efficacy might depend on hormone receptor status in five out of 10 SNPs with the smallest interaction *P* values, with a greater effect in the group of TNBC patients (Table 3). Patients with a homozygous common genotype and triple-negative tumors appeared to benefit from BEV treatment, whereas the other patients did not. For example, with regard to SNP rs1374749, the effect of the common genotype on the drug response was much greater in triple-negative patients (OR 3.20; 95% CI, 1.68 to 6.07) than in patients with hormone receptor–positive, HER2-negative tumors (OR 0.91; 95% CI, 0.45 to 1.87).

All of the logistic regression models were well calibrated. The *P* values in the Hosmer–Lemeshow tests ranged from 0.13 to 0.91, and the median value was 0.49.

Discussion

In this pre-planned biomarker substudy of the neoadjuvant GeparQuinto (GBG44) trial, germline genetic variants in VEGF pathway genes were associated with bevacizumab treatment response as assessed by pCR. Remarkably, out of top 10 SNPs with the smallest *P* values, 5 SNPs were located in *VEGFA*. None of these remained statistically significant after adjustment for multiple testing. The effect of some SNPs in *VEGF-A* appeared to be stronger in patients with triple-negative breast tumors.

A biomarker that is able to predict the efficacy of BEV is urgently needed, particularly for patients with breast cancer, as not all clinical trials have shown clear superiority for the drug. Several trials have assessed the predictive value of circulating VEGF-A levels in serum. Although high VEGF-A serum levels were consistently associated with poor prognosis, they failed as a predictive marker for BEV treatment in a meta-analysis including colorectal, lung, breast and renal cancer patients.[27] In addition, high expression levels of a *VEGF-A* splice variant in tumor tissue were associated with more favorable PFS and OS in metastatic breast cancer in a single BEV-treated group.[28] Also levels of the short isoform VEGF-A, which are currently being prospectively validated in a phase III metastatic breast cancer trial (MERiDiAN), look promising as a predictive biomarker for BEV.[29] However, despite these concerted

efforts using a wide range of biological sample types, a validated marker predictive of treatment outcome for BEV has so far not been identified.

In the present study, homozygous carriers of the rare rs833058 variant, which correspond to the AA genotype, showed the strongest association with benefit from BEV treatment. Additional predictive SNPs in VEGF-A were intronic (rs3025030), located in the promoter region (rs699947) or 3'UTR region (rs3025039) of *VEGF-A*. The rs833058 SNP is in linkage (r^2 = 0.51) with rs699947 and other SNPs in *VEGF-A*. Interestingly, AA genotype carriers of both rs833058 and rs699947 are associated with an upstream 18-nucleotide insertion,[30] which has previously been linked with low VEGF expression levels both *in vitro* and *in vivo*.[31, 32] In addition, expression quantitative trait locus (eQTL) analyses from publicly available data sources show an effect of rs699947 on *VEGF-A* expression in Caucasians (rho = 0.263; *P* = 0.0060)[33], thus confirming that this variant is linked to *VEGF-A* expression. Interestingly, the AA genotype of rs699947 was also associated with better OS in metastatic breast cancer patients when treated with a combination of BEV and paclitaxel, but not with paclitaxel alone (E2100 study).[19] The same genotype was associated with a better response to BEV in the present GeparQuinto study (OR 2.24; 95% CI: 1.31-3.84).

A recent comprehensive study analyzed 195 SNPs in six randomized phase III trials testing the addition of BEV to standard chemotherapy in colorectal, pancreatic, lung, renal, breast, and gastric cancer, involving a total of 716 BEV treated patients and 686 patients receiving placebo.[20] In this analysis, genetic variants in *VEGF-C, EPAS1, IL8RA,* but also *VEGF-A* were predictors of treatment outcome after BEV treatment.[20] Out of the 195 SNPs analyzed in this meta-analysis, we analyzed 110 SNPs in the GeparQuinto study, which included 729 BEV treated patients and 724 control patients. Our study thus has a comparable sample size, but has the advantage that it only consists of one cancer type evaluated for a uniform endpoint in a single randomized study. As such, GeparQuinto is the first study, in which it is possible to compare a comprehensive set of SNPs for a large set of patients. Intriguingly, when our results are compared with those obtained in the meta-analysis,[20] the same *VEGF-A* SNPs identified in GeparQuinto were also significant in the meta-analysis. In particular, AA carriers of rs833058 were also associated with prolonged PFS in the meta-analysis, whereas both rs3025030 and rs3025039 were significant at *P*<0.05 in the meta-analysis. Moreover, when focusing on the breast cancer trial included in this meta-analysis, i.e., the AVADO phase 3 trial for metastatic breast cancer, both rs699947 and rs3025039 were significant (*P*<0.05) in a treatment by interaction analysis.[20]

Interestingly, the SNPs in the 3' UTR region of *VEGF-A*, i.e., rs3025030 and rs3025039, also showed a differential effect in the molecular subgroup analysis, with a greater predictive effect in triple-negative patients. The *VEGF-A* SNPs close to the

promoter region did not show this effect. Bearing in mind that none of the interaction *P* values were significant after adjustment for multiple testing and that the effect of these SNPs on *VEGF* expression in unknown, this result suggests that the effects through which the SNPs in the promoter region and those in the 3' UTR region are mediated may differ from each other.

Finally, we also observed an association between rs7995976 in *FLT1* and pCR after BEV. Interestingly, increased sFLT1 levels have previously been linked with poor outcome after anti-angiogenic therapy in several studies,[34, 35] whereas a significant correlation between another genetic variant in *FLT1*, i.e., rs9582036, and outcome after BEV was observed in pancreatic and renal cell carcinoma patients. Since sFLT1 is an endogenous anti-angiogenic factor, tumors exposed to high levels of sFLT1 are indeed not expected to benefit from any additional exposure to anti-angiogenic molecules, such as BEV. Since the functional effect of rs7995976 on *FLT1* expression is unknown, it thus remains an outstanding question whether this variant should be considered a true-positive candidate biomarker for BEV treatment. In this context multi-tyrosinkinase inhibitors which target FLT1 might be of interest when assessing BEV response. There are already several clinical trials assessing the addition of sunitinib or sorafenib as a monotherapy after a combination of BEV with standard chemotherapy.[36, 37] Unfortunately, these studies were prematurely closed because of an unfavorable toxicity profile. Interestingly, studies have shown that EPAS1 (HIF2α) regulates soluble VEGF receptor-1 (sFLT1) production from macrophages, which contributes to the inhibition of VEGFA during angiogenesis in mouse models.[38] This mechanism might also explain why we observed an increased response to BEV treatment in homozygous carriers of the common (wild-type) alleles for 3 *EPAS1* SNPs.

Overall this study has several strengths and limitations. Firstly, although it is one of the largest single-study analyses from a prospectively randomized bevacizumab trial, the sample size of approximately 600 patients in each treatment arm and a total of 261 patients with pCR is still too small to identify genetic associations of modest effect size while correcting for multiple testing. As a result, none of the SNPs was statistically significant after adjustment for multiple testing. However, since several other studies involving breast cancer patients treated with BEV observed similar association signals, our results support the view that response to BEV is a complex genetic phenotype that requires additional studies to be carried out in this area, in order not to miss a signal that may have been overseen due to limited power.

In conclusion, we failed to observe a strong association between germline genetic variants in VEGF pathway genes and BEV treatment outcome. However, some SNPs in *VEGF-A,* which have been shown in other studies to correlate with overall survival and have been shown to be associated with reduced *VEGF-A* expression, were

predictive for BEV treatment. Additional studies are therefore warranted to explore the effects of these variants on bevacizumab treatment outcome.

Acknowledgments

Conflicts of interest

Alexander Hein has received honoraria from F. Hoffmann–La Roche for speaker engagement. Diether Lambrechts has received research funding from F. Hoffmann La Roche related to research into biomarkers for bevacizumab and is mentioned as inventor on patents describing some of these variants as potential biomarker for treatment outcome. Gunter von Minckwitz has received research funding from F. Hoffmann La Roche. Hans Tesch has received honoraria from F. Hoffmann–La Roche, GSK and Novartis. Claus Hanusch is member of speakers' bureau, Advisory Board Member and is conducting trials with financial support from F. Hoffmann–La Roche, Pfizer, Boehringer-Ingelheim, Amgen and Novartis. Jens Huober is Advisory Board Member for F. Hoffmann–La Roche and Novartis. Volkmar Müller has received honoraria from F. Hoffmann–La Roche for speaker engagement. Brigitte Rack has received honoraria from F. Hoffmann–La Roche. Peter A. Fasching has received honoraria from F. Hoffmann–La Roche, Amgen, Pfizer, Novartis and Genomic Health for speaker engagement. PAF is Advisory Board Member for F. Hoffmann–La Roche, Pfizer and Novartis and has received Research Funding from Amgen and Novartis. The other authors have no conflicts of interest to disclose regarding the publication of this paper.

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Table 1.   Patient characteristics and distribution of pCR

Characteristic	No pCR Mean (SD) or count (%)	pCR Mean (SD) or count (%)
Age (year)	49.9 (10.2)	47.0 (10.1)
BMI (kg/m²)	25.8 (4.8)	25.9 (4.7)
Treatment arm		
EC-T	606 (83.7)	118 (16.3)
ECB-TBEV	586 (80.4)	143 (19.6)
Lymph-node status		
cN+	710 (83.6)	139 (16.4)
cN0	482 (79.8)	122 (20.2)
Histology		
Ductal	944 (81.2)	219 (18.8)
Lobular	137 (94.5)	8 (5.5)
Other	111 (76.6)	34 (23.4)
Hormone receptor status		
Negative	318 (64.0)	179 (36.0)
Positive	874 (91.4)	82 (8.6)
Tumor stage		
cT1	174 (74.4)	60 (25.6)
cT2	800 (81.5)	182 (18.5)
cT3	76 (91.6)	7 (8.4)
cT4	142 (92.2)	12 (7.8)
Grading		
G1	50 (94.3)	3 (5.7)
G2	691 (89.6)	80 (10.4)
G3	451 (71.7)	178 (28.3)

BEV, bevacizumab; BMI, body mass index; EC, epirubicin and cyclophosphamide; pCR, pathological complete remission; SD, standard deviation; T, paclitaxel.

Table 2. Ten SNPs with the lowest *P* values for treatment by genotype interaction relative to pathological complete response. Odds ratios for bevacizumab versus standard treatment by genotype are shown

SNP	Major allele/ minor allele	Gene	Chr	Position	MAF	*P* value[1]	Odds ratio (95% confidence intervals)		
							Homozygous common	Heterozygous	Homozygous rare
rs833058	G/A	*VEGF-A*	6	43764117	0.39	0.003	2.36 (1.49, 3.75)	1.21 (0.89, 1.65)	0.62 (0.34, 1.13)
rs699947	A/C	*VEGF-A*	6	43768652	0.50	0.032	2.24 (1.31, 3.84)	1.40 (1.04, 1.88)	0.87 (0.53, 1.45)
rs3025039	C/T	*VEGF-A*	6	43784799	0.17	0.040	1.71 (1.20, 2.44)	0.97 (0.62, 1.53)	0.55 (0.22, 1.41)
rs3025030	G/C	*VEGF-A*	6	43782850	0.16	0.041	1.72 (1.20, 2.45)	0.98 (0.62, 1.54)	0.56 (0.22, 1.42)
rs7995976	C/A	*FLT1*	13	28366923	0.25	0.049	1.06 (0.72, 1.56)	1.73 (1.18, 2.53)	2.82 (1.28, 6.19)
rs4953344	T/C	*EPAS1*	2	46325319	0.18	0.058	1.65 (1.15, 2.35)	0.95 (0.59, 1.54)	0.55 (0.21, 1.48)
rs3768728	T/C	*EPAS1*	2	46363652	0.15	0.067	1.61 (1.14, 2.27)	0.90 (0.52, 1.56)	0.50 (0.16, 1.54)
rs1374749	G/A	*EPAS1*	2	46369294	0.46	0.073	1.88 (1.16, 3.03)	1.28 (0.94, 1.72)	0.87 (0.50, 1.51)
rs3025033	A/G	*VEGF-A*	6	43783338	0.18	0.074	1.68 (1.17, 2.41)	1.05 (0.68, 1.61)	0.65 (0.27, 1.58)
rs1629140	G/A	*EGLN3*	14	33944620	0.42	0.103	1.84 (1.16, 2.93)	1.30 (0.96, 1.76)	0.92 (0.52, 1.61)

[1] Uncorrected *P* value for treatment by genotype interaction based on multiple logistic regression model with the additional predictors age, body mass index, tumor stage, lymph-node status, histology, grading, and hormone receptor status. None of the Bonferroni–Holm corrected *P* values were significant: *P* = 0.34 for rs833058 and *P* = 1.00 for all other SNPs.

Chr, chromosome; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

Table 3.   Hormone receptor–specific associations between treatment, genotype, and pathological complete response. Odds ratios for bevacizumab versus standard treatment by genotype are shown

SNP[1]	HR	Odds ratio (95% confidence intervals)		
		Homozygous common	Heterozygous	Homozygous rare
rs3025039	HR–	2.17 (1.38, 3.41)	1.04 (0.56, 1.93)	0.50 (0.14, 1.77)
(*VEGFA*)	HR+	1.21(0.67, 2.16)	0.89 (0.47, 1.68)	0.66 (0.18, 2.40)
rs3025030	HR–	2.24 (1.42, 3.53)	1.07 (0.58, 1.99)	0.52 (0.15, 1.82)
(*VEGFA*)	HR+	1.16 (0.65, 2.07)	0.86 (0.46, 1.63)	0.64 (0.18, 2.33)
rs3768728	HR–	2.36 (1.50, 3.70)	0.81 (0.39, 1.68)	0.28 (0.06, 1.23)
(*EPAS1*)	HR+	0.91 (0.53, 1.57)	1.06 (0.46, 2.44)	1.22 (0.22, 6.86)
rs1374749	HR–	3.20 (1.68, 6.07)	1.55 (1.05, 2.28)	0.75 (0.37, 1.52)
(*EPAS1*)	HR+	0.91 (0.45, 1.87)	1.00 (0.62, 1.61)	1.09 (0.44, 2.71)
rs3025033	HR–	2.21 (1.39, 3.51)	1.14 (0.63, 2.04)	0.59 (0.18, 1.96)
(*VEGFA*)	HR+	1.15 (0.64, 2.09)	0.90 (0.49, 1.63)	0.70 (0.21, 2.34)

[1] Subset of SNPs from Table 2. Only those SNPs were included for which the logistic regression model with hormone receptor interaction terms a had better (i.e., smaller) Akaike information criterion than the logistic regression model without hormone receptor interaction terms.

Abbreviations: HR, hormone receptor; SNP, single nucleotide polymorphism.

Figure 1.   The *VEGFA* gene with –log of *P* values for genes in the area of *VEGFA* (figure generated with LocusZoom [39])