Genetic predisposition to bevacizumab-induced hypertension

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HIGHLIGHTS

• Variation in WNK1, KLKB1, and GRK4 may be associated with BIH.
• WNK1, KLKB1, and GRK4 are biologically plausible mediators of BIH.
• A composite risk model identified 43% of patients who developed BIH.

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ABSTRACT

Objective. Bevacizumab, a monoclonal antibody to VEGF, has shown efficacy in ovarian, cervical and endometrial cancer in addition to several other solid tumors. Serious side effects include hypertension, proteinuria, bowel perforation, and thrombosis. We tested the hypothesis that genetic variation in hypertension-associated genes is associated with bevacizumab-induced hypertension (BIH).

Methods. Patients with solid tumors treated with bevacizumab in combination with other therapy were identified from six clinical trials. Haplotype-tagging (ht) SNPs for 10 candidate genes associated with hypertension were identified through the International Hapmap Project. Germline DNA was genotyped for 103 htSNPs using mass spectrometry. Bevacizumab toxicities were identified from clinical trial reports. Haplotypes were reconstructed from diploid genotyping data and frequencies were compared using standard two-sided statistical tests.

Results. The study included 114 patients with breast, lung, ovarian, or other cancers, of whom 38 developed BIH. WNK1, KLKB1, and GRK4 were found to contain single loci associated with BIH. Haplotype analysis of WNK1, KLKB1, and GRK4 identified risk haplotypes in each gene associated with grade 3/4 BIH. A composite risk model was created based on these haplotypes. Patients with the highest risk score were the most likely to develop grade 3/4 BIH (OR = 6.45; P = 0.005; 95%CI, 1.86–22.39).

Conclusions. We concluded that genetic variation in WNK1, KLKB1, and GRK4 may be associated with BIH. These genes are biologically plausible mediators due to their role in blood pressure control, regulating sodium homeostasis and vascular tone. This preliminary risk model performed better than population-based risk models and when further validated may help risk-stratify patients for BIH prior to initiating therapy.

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1. Introduction

Bevacizumab is a recombinant monoclonal antibody to vascular endothelial growth factor (VEGF). VEGF binds to endothelial cell receptors and promotes tumor angiogenesis by encouraging endothelial cell proliferation, migration and survival, and increasing vascular permeability. The efficacy of bevacizumab as an anti-cancer agent relies on its inhibition of the angiogenesis-promoting biological functions of VEGF [1]. Bevacizumab is currently FDA approved for the treatment of cervical cancer [2], ovarian cancer [3–5], colorectal cancer [6], glioblastoma [7], non–small-cell lung cancer [8,9], renal cell cancer [10] and has shown activity in many other tumors including endometrial [11], breast [12], soft tissue sarcomas [13] and malignant mesothelioma [14] as a single agent or in combination with cytotoxic agents.

Studies of bevacizumab have demonstrated that inhibition of VEGF induces or exacerbates hypertension in some patients and can cause other serious side effects including thrombosis, wound-healing complications, hemorrhage, gastrointestinal perforation, and proteinuria.

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According to a meta-analysis of 12,656 patients with a variety of tumors receiving bevacizumab, the incidence of hypertension was 23.6%. The incidence of severe hypertension, defined as National Cancer Institute Common Toxicity Criteria grade 3 or 4, was 7.9%, and the relative risk for developing severe hypertension was 5.3. This increased risk was observed with both low- and high-dose bevacizumab regimens [15]. Another meta-analysis reviewing 9062 patients receiving bevacizumab found the relative risk of all-grade hypertension to be 5.3 [16]. Other studies have reported rates of severe hypertension in patients receiving bevacizumab ranging from 15 to 25% [17,18]. The mechanism of bevacizumab-induced hypertension remains unclear.

Scientific advances have altered the study of the genetic epidemiology of complex diseases. The International HapMap Project database provides a catalog of the common human genetic variants across several populations [19,20]. These data have resulted in a shift away from linkage analysis towards association mapping of genes that affect complex phenotypes. HapMap has catalogued the block structure, or haplotype pattern, of the human genome. By exploiting the underlying patterns of linkage disequilibrium (LD), it is possible to use haplotype-based association studies to identify disease susceptibility alleles [21]. Specific haplotype blocks may contain genetic variants involved in susceptibility to disease [22]. Haplotype analysis has resulted in the publication of a series of studies that examine potential genetic contributions to common diseases, including prostate cancer, breast cancer, diabetes, and coronary artery disease [23]. Furthermore, haplotype analysis has been used to predict medication side effects [24].

Association studies are especially useful for complex disorders like hypertension, in which multiple genetic factors interact with the environment to determine phenotype. Familial and epidemiological studies suggest that 30–50% of blood pressure variation is genetic in origin [25]. Genes involved in complex diseases, like hypertension, have been discovered through linkage mapping. This method successfully identified genes for several rare monogenic forms of blood pressure deregulation, but no single gene has been found to have a major effect on blood pressure variation in the general population. However, these Mendelian hypertensive disorders highlight potential pathways and mechanisms of hypertension and provide candidate genes for genetic association research [26]. Genetic variation in several blood pressure deregulation-associated genes has been associated with hypertension [27–30].

Almost one third of patients treated with bevacizumab develop hypertension, which might imply that common variation present in the population contributes to the susceptibility for this toxicity. Previous studies have examined genetic variation in hypertension-associated candidate genes and identified polymorphisms and haplotypes associated with essential hypertension. The purpose of the study was to test the hypothesis that genetic variation in hypertension-associated genes is associated with the risk for developing bevacizumab-induced hypertension.

### 2. Methods

#### 2.1. Study population

Cases and controls were collected from one of six ongoing or completed IRB-approved protocols at Memorial Sloan-Kettering Cancer Center (Supplementary Table S1). This study was also specifically approved by the local IRB and written consents were obtained from all patients. In these protocols, bevacizumab is used in combination with chemotherapy agents, molecularly targeted agents, or hormonal agents for patients with breast cancer, non–small-cell lung cancer, serous ovarian cancer, and other advanced solid tumors. All patients were white as a result of the demographics of the patient population treated at this institution during the study time period and to limit genetic heterogeneity. Hypertension was graded according to the National Cancer Institute Common Toxicity Criteria version 3.0 (ctepcancer.gov) and recorded as part of the protocol. Patients with existing uncontrolled hypertension at time of enrollment, defined as systolic blood pressure > 150 or diastolic blood pressure > 90, were excluded from the trial. Hypertension that developed while on treatment protocols was managed by protocol guidelines outlined in Supplementary Table 2. All patients included in this study had undergone surgical resection or excisional biopsy of their disease. Benign tissue removed at the time of surgery was used for DNA extraction. Clinical information was extracted from institutional, research, and laboratory databases.

#### 2.2. Candidate genes and SNP selection

Eleven candidate genes were identified from a review of the literature on hypertension and genetic variation that contributes to blood pressure regulation (Table 1). These genes fit into one of four categories based on the mechanism of blood pressure regulation: genes involved in the renin-angiotensin-aldosterone system, genes related to bradykinin, genes involved in sodium regulation, and VEGF, the gene implicated in the activity of bevacizumab. We identified single nucleotide polymorphisms (SNPs) within these genes from www.hapmap.org. Polymorphisms were selected for each gene provided that the minor allele frequency was >0.05 in the CEPH population (Utah Residents with Northern and Western European Ancestry, CEU). Haplotype-tagging SNPs (htSNPs) were selected using Tagger (www.broadinstitute.org/mpg/tagger) to capture the unmeasured SNPs with a minor allele frequency ≥0.05 and r² ≥ 0.8. One hundred and ten SNPs in 11 genes were initially selected for analysis (Supplementary Table S2). SNPs below a genotyping call rate of 95% were removed (n = 7), leaving 103 SNPs in 11 genes for statistical analysis.

#### 2.3. Genotyping and quality control

Genomic DNA was prepared from peripheral lymphocytes using QIAGEN DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, CA).

### Table 1

Candidate hypertension-associated genes.

| Renin-angiotensin-aldosterone system | Angiotensinogen (AGT) | Angiotensin II Type I Receptor (AGTR1) | Aldosterone Synthase (CYP11B2) | Angiotensin-I-converting Enzyme (ACE) |
| Bradykinin-related | Bradykinin Receptor 1 (BDKRB1) | Kallikrein (KlkR1) | Sodium Regulation | WNK1 (with no lysine K) |
| | G Protein-coupled Receptor Kinase 4 (GRK4) | Epithelial Sodium Channel (ENaC-alpha/SCNN1a) | G Protein B3 Subunit (GNB3) | Vascular Endothelial Growth Factor (VEGF) |

### Table 2

Patient characteristics.

<table>
<thead>
<tr>
<th>Gender</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>19 (17)</td>
</tr>
<tr>
<td>Female</td>
<td>95 (83)</td>
</tr>
<tr>
<td>Disease site</td>
<td>n (%)</td>
</tr>
<tr>
<td>Breast</td>
<td>55 (48)</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>25 (22)</td>
</tr>
<tr>
<td>Serous ovarian cancer</td>
<td>19 (17)</td>
</tr>
<tr>
<td>Advanced solid tumor</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Hypertension grade</td>
<td>n (%)</td>
</tr>
<tr>
<td>Grades I–IV</td>
<td>38 (33)</td>
</tr>
<tr>
<td>Grade I</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Grade II</td>
<td>18 (16)</td>
</tr>
<tr>
<td>Grade III</td>
<td>13 (11)</td>
</tr>
<tr>
<td>Grade IV</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

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TaqMan® allelic discrimination assays performed on an ABI prism 7900 were genotyped using Duplicate samples and negative controls were included in each plate. Calls were evaluated and edited manually by peak and cluster analysis. Genotypes were called with SpectroTyper (Sequenom). Onto a SpectroCHIP (Sequenom) and separated by MALDI-TOF mass spectrometry. Genotyping was performed using Sequenom iPLEX matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) mass spectrometry. PCR assays and single base extension (SBE) primers for the htSNPs were designed using the MassARRAY AssignDesigner software, version 3.0 (Sequenom, San Diego, CA). PCR and SBE oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA). Multiplex pools contained between 10 and 24 combined SNP assays. Following PCR amplification, products were treated with shrimp alkaline phosphatase to neutralize un-incorporated nucleotide tri-phosphates. The SBE reaction was initiated with iPLEX enzyme and mass-modified terminators. Synthetic oligonucleotides bind adjacent to the site of the target SNP and incorporate nucleotides complementary to the template. The SBE products were desalted, spotted for mass spectrometry. PCR and SBE oligonucleotides for the incorporation of the target SNP, generating products with allele-specific differences. The SBE reaction was initiated with iPLEX enzyme and mass-modified terminators. Synthetic oligonucleotides bind adjacent to the site of the target SNP and incorporate nucleotides complementary to the template. Mass-modified nucleotides terminate the extension reaction after the incorporation of the target SNP, generating products with allele-dependent mass differences. The SBE products were desalted, spotted onto a SpectroCHIP (Sequenom) and separated by MALDI-TOF mass spectrometry. Genotypes were called with SpectroTyper (Sequenom). Calls were evaluated and edited manually by peak and cluster analysis. Duplicate samples and negative controls were included in each plate.

For quality assurance, four representative SNPs (rs2158501, rs11064560, rs1912826, and rs4253331) were genotyped using TaqMan® allelic discrimination assays performed on an ABI prism 7900 sequence detection system using high-fidelity reagents. Primers and fluorescently labeled MGB-NFQ probes were designed and synthesized by Applied Biosystems (Foster City, CA). Assays were performed in a 384-well format with approximately 20 ng of genomic DNA, 2.5 μL of 2× Taqman Universal PCR Master Mix, 0.125 μL of 40× Taqman probes/primers mix, and 2.375 μL H2O in a total reaction volume of 5 μL.

Thermocycling conditions were 95 °C for 10 min followed by 40 cycles of 92 °C for 15 s and 62 °C for 1 min. Fluorescence data were collected during a post-PCR plate read and analyzed using the allelic discrimination SDSv2.1 program (Applied Biosystems). The overall concordance between Sequenom and Taqman genotyping was 99% (Supplementary Table S3).

### 2.4. Statistical analysis

All SNPs were tested and found to be in Hardy–Weinberg equilibrium at P > 0.1. The chi-square and the Cochran-Armitage trend tests were used to compare single loci genotypes between patients who developed hypertension of all grades (1–4) during treatment with bevacizumab and patients who did not. Bayesian statistical methods were used to reconstruct haplotypes from diploid genotype data using PHASE, version 2 (stephenslab.uchicago.edu/software.html) [31,32]. Haplotypes were compared using Fisher’s exact test.

Genotype power calculations were performed using the genetic power calculator of Purcell et al. (pngu.mgh.harvard.edu/~purcell/gpc) [33]. Assuming a disease prevalence of 0.20, a relative risk of 1.5, a minor allele frequency of 0.3, at an alpha of 0.05 this sample size has an estimated power of 80% using a multiplicative genetic model. Permutation testing was used to identify significant differences in overall haplotype frequencies between cases and controls. In the haplotype analysis, bevacizumab-induced hypertension was defined as grade 3 or 4 hypertension according to the NCI Common Toxicity Criteria version 3.0.

### 3. Results

#### 3.1. Clinical characteristics

The 114-patient study population consisted of 19 males and 95 females with a mean age of 57 ± 11.6 years (range, 29–86). Fifty-five patients were treated for breast cancer, 25 for non–small-cell lung cancer, 19 for serous ovarian cancer, and 15 for other advanced solid tumors. Thirty-eight (33%) patients developed grades 1–4 hypertension during treatment with bevacizumab, and 14 (12%) developed severe, grade 3 or 4 hypertension (Table 2). Three patients also developed proteinuria.

#### 3.2. Single loci testing

Of the 11 hypertension-associated candidate genes, WNK1, KLKB1, and GRK4 had individual htSNPs associated with bevacizumab-induced hypertension. Single loci testing identified 3 of 22 WNK1 htSNPs—rs11064519, rs11064560, and rs2158501—individually associated with grades 1–4 bevacizumab-induced hypertension (P < 0.01; allelic test). Single loci testing also identified association with KLKB1 and GRK4 SNPs—rs1912826 and rs1419044—at P < 0.01 and < 0.001, respectively (Table 3). Allele frequencies are reported in Supplementary Table S4.

#### 3.3. Haplotype analysis

Haplotype analysis identified 11 common haplotypes for WNK1, 7 for KLKB1, and 9 for GRK4 (Supplementary Table S5). Combined risk haplotypes were compared to non-risk haplotypes to test for associations with grade 3 or 4 bevacizumab-induced hypertension. Haplotype analysis of WNK1 identified three risk haplotypes—haplotypes 2, 3, and 8 (OR = 3.9; P = 0.01; 95% CI, 1.3–11.8). For KLKB1, haplotypes 2, 3, and 4 were associated with bevacizumab-induced hypertension and these risk haplotypes had an OR of 2.19, although this finding was not statistically significant.
significant ($P = 0.055$; 95% CI, 0.97–4.95). For GRK4, haplotypes 4, 5, and 9 were associated with the lack of bevacizumab-induced hypertension (OR = NA; $P = 0.0035$) (Table 4).

### 3.4. Bevacizumab-induced hypertension risk model

A risk model was created from the three genes individually associated with bevacizumab-induced hypertension—WNK1, KLKB1, and GRK4. A risk score is generated based on the presence of hypertension-associated haplotypes. One or more copies of a hypertension-associated haplotype in each of these three genes is assigned 1 point (range, 0–3). Patients with the highest risk score have at least one hypertension-associated haplotype in each of WNK1, KLKB1, and GRK4, and are assigned a total of 3 points. Patients with the lowest risk score have no hypertension-associated haplotype in WNK1, KLKB1, and GRK4, and are assigned 0 points. Patient scores in the highest risk category were associated with the development of severe hypertension when treated with bevacizumab (OR = 6.45; $P = 0.005$; 95% CI, 1.86–22.39) (Table 5). Ten patients had the highest risk score and did not develop hypertension. The specificity and positive predictive value for this model are 43% (95% CI, 31%–79%) and 38% (95% CI, 18%–65%), respectively. The specificity and negative predictive value of this model are 90% (95% CI, 82%–95%) and 91% (95% CI, 84%–96%), respectively (Table 6).

### 4. Discussion

We found that genetic variation in three genes, WNK1, KLKB1, and GRK4, is associated with bevacizumab-induced hypertension. The association between these three blood pressure-related genes and hypertension is supported by evidence from several studies. WNK1 is a gene that encodes a serine-threonine kinase that contributes to blood pressure homeostasis through regulation of the sodium chloride co-transporter in the distal convoluted tubule [34]. Mutations in WNK1 cause Gordon’s syndrome, a rare Mendelian disorder characterized by hypertension and hyperkalemia. Recent studies of genetic variation in WNK1 have identified polymorphisms associated with hypertension in large family-based samples of Caucasian patients [35,36] and three common polymorphisms that are associated with ambulatory blood pressure and response to thiazide diuretics in African-American and Caucasian cohorts [37]. KLKB1 encodes plasma kallikrein, a serine protease that catalyzes the release of kinins and other vasoactive peptides and might be implicated in the pathogenesis of hypertension. Research on genetic variation in KLKB1 has identified two SNPs and two haplotypes associated with the risk of essential hypertension in a northern Han Chinese population [38] and found that KLKB1 polymorphisms have significant trait association with activation of the renin-angiotensin system [39]. GRK4 encodes a member of the guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily of the serine threonine protein kinase family. Dopamine regulates blood pressure through G protein-coupled receptors (GPCRs) in the proximal tubule and thick ascending limb of Henle that inhibits sodium reabsorption. These GPCRs are in turn regulated by GRK proteins. GRK4 phosphorylates GPCRs, uncoupling the dopamine receptor D1 receptor from its G-protein-effector complex, which interferes with sodium excretion. Previous work has found that allelic variants in GRK4 are associated with essential hypertension [30,40].

Our study has found that both SNPs and haplotypes in WNK1, KLKB1, and GRK4 are associated with the development of bevacizumab-induced hypertension. WNK1 and KLKB1 were found to each have three haplotypes associated with bevacizumab-induced hypertension. When haplotypes from these three genes were combined to create a risk model, scores in the highest risk category were associated with the development of severe hypertension (grade 3 or 4) when treated with bevacizumab. This model, therefore, correctly identifies approximately 40% of the individuals who may develop bevacizumab-induced hypertension that requires clinical intervention, which is considerably better than population-based risk models, which would randomly predict approximately 15% of grade 3 or 4 hypertension seen in the general population treated with bevacizumab. Currently monitoring protocols for bevacizumab-induced hypertension vary between centers. Some institutions and physicians recommend that all patients being treated with bevacizumab monitor their blood pressure regularly while at home while others do not have specific guidelines apart from routine blood pressure evaluation at clinical visits. A test that could predict risk of developing BH could help with patient counseling and monitoring recommendations. Furthermore, considering the controversy surrounding the clinical utility of bevacizumab, a model like the one presented here may help with the decision-making process for select patients.

This study has multiple limitations. The study population was small and included heterogeneity in gender and primary tumor type. Furthermore, bevacizumab was combined with various additional drugs in each protocol and the drug combination may contribute to the development of side effects like hypertension. Finally, the management of bevacizumab-induced hypertension was not uniform between trials. Further validation studies in a population with less heterogeneity are necessary to achieve a better understanding of BH.

Though FDA approved for the treatment of ovarian cancer, cervical cancer, colorectal cancer, glioblastoma, non–small-cell lung cancer and renal cell cancer, bevacizumab is currently being investigated and demonstrating efficacy in many other cancers, including endometrial cancer. Nonetheless, there are serious academic and financial controversies regarding the role of bevacizumab in the treatment of various malignancies. Despite ongoing pharmacogenetic investigations of genetic variation that contribute to bevacizumab biologic activity, there has been minimal study of genetic polymorphisms as they relate to side effects, including drug-induced hypertension. The potential clinical benefit of this model is the ability to stratify patients into risk groups for developing hypertension prior to administration of the drug. Patients in the highest risk group could be monitored more closely for changes in blood pressure throughout treatment, allowing hypertension to be detected and treated without delay.

In summary, this study explores the association between genetic variation in hypertension-associated genes and predisposition to bevacizumab-induced hypertension. We have identified three biologically plausible genes associated with the development of hypertension during treatment with bevacizumab for various advanced solid tumors. This preliminary risk model may better predict which patients will develop clinically important hypertension compared with a population-based risk assessment.

### Table 5

<table>
<thead>
<tr>
<th>Gene or model</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNK1</td>
<td>3.9</td>
<td>1.3–11.8</td>
<td>0.01</td>
</tr>
<tr>
<td>KLKB1</td>
<td>2.19</td>
<td>0.97–4.95</td>
<td>0.055</td>
</tr>
<tr>
<td>GRK4*</td>
<td>NA</td>
<td>NA</td>
<td>0.0035</td>
</tr>
<tr>
<td>Composite model</td>
<td>6.45</td>
<td>1.86–22.39</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* OR not accurate due to empty cell, but ~8.

### Table 6

Risk scoring system for severe bevacizumab-induced hypertension (grades 3/4).

<table>
<thead>
<tr>
<th>Risk score</th>
<th>No hypertension, n (%)</th>
<th>Hypertension, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2 points (low risk)</td>
<td>86 (90.6)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>3 points (high risk)</td>
<td>10 (10.4)</td>
<td>6 (42.9)</td>
</tr>
</tbody>
</table>

$P = 6.45$ (OR = 0.005; 95% CI, 1.86–22.39).
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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jygyno.2017.09.017.

References