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ORIGINAL PAPER

Genetic markers of bevacizumab-induced hypertension

Diether Lambrechts · Matthieu Moisse · Paul Delmar · David W. Miles · Natasha Leighl · Bernard Escudier · Eric Van Cutsem · Aruna T. Bansal · Peter Carmeliet · Stefan J. Scherer · Sanne de Haas · Celine Pallaud

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Abstract
Purpose There are currently no validated biomarkers predicting bevacizumab treatment outcome or toxicity. We combined biomarker data from six phase III trials of bevacizumab to assess whether genetic variation in vascular endothelial growth factor-A (VEGF-A) pathway or hypertension-related genes are associated with bevacizumab-induced hypertension.
Experimental design Germline DNA was available from 1,631 patients receiving bevacizumab-containing therapy for advanced solid tumors. Overall, 194 white patients had grade 1–4 bevacizumab-induced hypertension. In total, 236 single nucleotide polymorphisms (SNPs) located in *VEGF-A*, *VEGF-A* receptors (*FLT1* and *KDR*), and other genes were selected using a SNP tagging approach and genotyped. A logistic regression on individual patient data was performed after adjustment for cancer type and five other covariates.
Results Ten SNPs were associated with bevacizumab-induced hypertension ($P \leq 0.05$), but none surpassed the threshold adjusted for multiple testing ($P < 0.0002$). The most significant *VEGF-A* pathway SNP was rs1680695 in *EGLN3* [allelic odds ratio (OR) 1.50 [95 % confidence interval (Cl) 1.09–2.07], $P = 0.012$]. Two additional SNPs, rs4444903 in *EGF* and rs2305949 in *KDR*, were associated with hypertension (allelic OR 1.57 [95 % CI 1.17–2.11], $P = 0.0025$; allelic OR 0.62 [95 % CI 0.42–0.93], $P = 0.020$, respectively) and closely linked to nearby functional variants. Consistent with previous reports, rs11064560 in *WNK1* was also associated with bevacizumab-induced hypertension (OR 1.41 [95 % CI 1.04–1.92], $P = 0.028$).
Conclusions The genes described in this large genetic analysis using pooled datasets warrant further functional

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D. Lambrechts (✉) · M. Moisse · P. Carmeliet
Vesalius Research Center, VIB, Campus Gasthuisberg, Herestraat 49, Box 912, 3000 Leuven, Belgium
e-mail: Diether.Lambrechts@vib-kuleuven.be

D. Lambrechts · M. Moisse
Laboratory of Translational Genetics, Department of Oncology, University of Leuven (KU Leuven), Leuven, Belgium

P. Delmar · S. de Haas · C. Pallaud
F. Hoffmann-La Roche, Basel, Switzerland

D. W. Miles
Mount Vernon Cancer Centre, Northwood, UK

N. Leighl
Department of Medicine, Princess Margaret Hospital, Toronto, ON, Canada

B. Escudier
Institut Gustave Roussy, Villejuif, France

E. Van Cutsem
Digestive Oncology, University Hospitals Leuven, Leuven, Belgium

A. T. Bansal
Acclarogen Ltd, St John's Innovation Centre, Cambridge, UK

S. J. Scherer
Genentech Inc, South San Francisco, CA, USA

investigation regarding their role in mediating bevacizumab-induced hypertension.

Keywords Anti-angiogenesis · Bevacizumab · Hypertension · Single nucleotide polymorphism · Predictive biomarker

Introduction

Bevacizumab is a humanized monoclonal antibody against vascular endothelial growth factor (VEGF)-A, a key factor inducing the formation of blood vessels (angiogenesis) in tumors [1]. Bevacizumab was the first anti-angiogenic agent approved for use in the clinic. In combination with standard chemotherapy, it is currently used for the treatment of metastatic colorectal cancer (mCRC) [2–4] and non-small cell lung cancer (NSCLC) [5]. The drug is also approved in combination with interferon α-2a for renal cell carcinoma (RCC) [6]. In addition, in Europe bevacizumab is approved with standard chemotherapy for advanced breast cancer [7, 8] and advanced ovarian cancer [9–11], while in the United States it is approved as a single agent for recurrent glioblastoma [12].

Treatment-related hypertension has been linked with several anti-angiogenic therapies, including bevacizumab [13–15] Hypertension is one of the most common side effects of bevacizumab therapy. According to a meta-analysis of 12,656 patients with cancer participating in phase II/III trials of bevacizumab, the incidence of all-grade bevacizumab-induced hypertension was 23.6 % [16]; grade 3/4 hypertension occurred in 7.9 % of patients. In individual phase III trials, incidences of grade 3/4 hypertension up to 17 % were reported with bevacizumab [7, 11, 17]. As early initiation of antihypertensive therapy may help to maintain treatment schedules [18, 19] and reduce complications [20, 21], identification of patients at high risk for bevacizumab-induced hypertension could be clinically valuable. Anti-angiogenic strategies are used increasingly for prolonged periods and/or in multiple treatment lines [4], and consequently the clinical importance of managing complications resulting from chronic blood pressure elevation might gain increased prominence.

One widely held hypothesis for the mechanism of angiogenesis inhibitor-associated hypertension centers on the role of VEGF-A in nitric oxide (NO) regulation [22, 23]. NO is a potent vasodilator that plays a critical role in blood pressure control. VEGF-A increases steady-state endothelial NO synthase (eNOS) expression in a time- and concentration-dependent manner [24]. In models of ischemic cardiomyopathy, hypotension is one of the major side effects of VEGF-induced angiogenesis. In the clinical setting, VEGF-A infusion causes rapid NO release and hypotension [25]. Conversely, inhibition of VEGF-A in animal studies reduces eNOS expression, leading to vasoconstriction and hypertension [26]. VEGF-A blockade and subsequent NO inhibition also appear to contribute to a state of endothelial dysfunction [21]. VEGF-A inhibition induces the regression of non-fenestrated microvessels—a phenomenon known as vascular rarefraction [27].

Given the frequency of bevacizumab-induced hypertension, common genetic variability may play a critical role in patients' susceptibility to hypertension and the severity of this toxicity. Genetic variability in *VEGF-A* pathway genes, which are important modulators of the NO pathway and vasculature tone in general, has broadly been proposed as a potential biomarker predictive of bevacizumab-induced hypertension [28]. Additionally, genetic variation in hypertension-associated genes (such as *NOS3*) may increase baseline blood pressure and render patients more susceptible to subsequent development of bevacizumab-induced hypertension. However, investigation of potential associations in studies requires large numbers of bevacizumab-treated patients. Therefore, we combined data from six randomized phase III clinical trials of bevacizumab and assessed whether genetic variants in the *VEGF-A* pathway or in hypertension-associated genes may act as biomarkers for bevacizumab-induced hypertension.

Patients and methods

Study design

The genetic analyses were performed on a subset of patients who consented to participate in a genetic substudy, donated a blood sample from which DNA could be successfully extracted and genotyped, and self-reported 'white' ethnicity (with the aim of limiting false positives by using an ethnically homogeneous patient population). Germline DNA was available from white patients treated in six placebo-controlled phase III clinical trials: (1) NO16966 (ClinicalTrials.gov identifier NCT00069095), which tested capecitabine–oxaliplatin versus 5-fluorouracil/folinic acid–oxaliplatin with or without bevacizumab as first-line treatment for mCRC [3]; (2) AViTA (ClinicalTrials.gov identifier NCT01214720), which tested the addition of bevacizumab to gemcitabine and erlotinib as first-line treatment for metastatic pancreatic cancer [29]; (3) AVAiL (ClinicalTrials.gov identifier NCT00806923), which tested the addition of bevacizumab to standard first-line chemotherapy (cisplatin–gemcitabine) for advanced/recurrent non-squamous NSCLC [30, 31]; (4) AVOREN (ClinicalTrials.gov identifier NCT00738530), which tested the addition of bevacizumab to interferon α-2a as first-line treatment for metastatic clear-cell RCC [6, 32]; (5)

AVADO (ClinicalTrials.gov identifier NCT00333775), which tested the addition of bevacizumab to docetaxel as first-line treatment for metastatic human epidermal growth factor receptor 2-negative breast cancer [33]; and (6) AVAGAST (ClinicalTrials.gov identifier NCT00548548), which tested the addition of bevacizumab to capecitabine–cisplatin chemotherapy as first-line treatment for advanced gastric cancer [34].

Trial protocols and genetic biomarker studies were approved by the institutional review board at each site and were in accordance with the Declaration of Helsinki, US Food and Drug Administration Good Clinical Practice, and local ethics and legal requirements. All patients included in this study provided separate written informed consent for genetic biomarker testing. Hypertension adverse events were graded in all six trials according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0) and recorded as a clinical outcome measure. All treatment-related grade 1–4 hypertension events reported at any time during the study period for each trial were included in the analysis because restriction to grade 3/4 hypertension provided insufficient events for statistically meaningful analysis.

Single nucleotide polymorphism analyses

We selected single nucleotide polymorphisms (SNPs) in the following genes involved in the VEGF-A pathway: *VEGF-A*, the *VEGF-A* homologs (placental growth factor [*PLGF*], *VEGF-B*, *VEGF-C*, and *VEGF-D* [also known as c-fos-induced growth factor or *FIGF*]), *VEGF* receptor-1 (*VEGFR-1* or *FLT1*), *VEGF* receptor-2 (*VEGFR-2* or *KDR*), *VEGF* receptor-3 (*VEGFR-3* or *FLT4*), regulators of hypoxia (hypoxia-inducible factor 1α [*HIF1A*], HIF-2α [*EPAS1*], factor inhibiting HIF-1α [*FIH1*], and von Hippel–Lindau tumor suppressor [*VHL*]), and the oxygen sensors (prolyl hydroxylase domain-containing protein 1 [*EGLN2*], 2 [*EGLN1*], and 3 [*EGLN3*]). Overall, we selected 211 tagging SNPs in these genes, as well as 9 SNPs previously correlated with bevacizumab outcome [35]. A detailed description of how SNPs were selected has been published previously [36, 37]. Finally, we also included 16 SNPs known to increase susceptibility to hypertension and thrombosis [38–41]. Overall, 236 SNPs were selected for genotyping.

Peripheral blood was sampled in K2EDTA Vacutainer tubes (BD, Franklin Lakes, New Jersey), and germline DNA was extracted from the precipitated leukocyte cell fraction. Genotyping for a subset of SNPs was carried out in a blinded manner at the Roche Translational Research Sciences Genetics Laboratories (Basel, Switzerland) using allele-specific polymerase chain reaction amplification, Sanger sequencing, and fragment-analysis platforms (AVAiL, AViTA, AVOREN, AVADO, NO16966, and AVAGAST). Genotyping for the majority of SNPs was done at the Vesalius Research Center, Leuven, Belgium, with MassARRAY iPLEX Gold (Sequenom, San Diego, California) as described previously [42]. Overall, 222 SNPs (94 %) were successfully genotyped in at least two studies. Of these 222 SNPs, five occurred at a frequency of less than 0.1 in the overall study population and were therefore excluded.

Statistical methods

We assessed Hardy–Weinberg equilibrium in white patients for the 217 SNPs included in this analysis using a standard χ^2 test with one degree of freedom. We detected seven markers with $P < 0.0002$; however, they were not excluded because patients were treated in various multicenter trials involving numerous countries. Instead, homogeneity of allele frequencies across trials was assessed using a χ^2 test of homogeneity of proportions. Seven of the 217 markers showed significant departure from homogeneity ($P < 0.0002$) and were excluded from association analyses. For the remaining 210 markers (Supplementary Tables 1 and 2), a meta-analysis of individual patient data was performed, after adjustment for cancer type and the following covariates: geographic region, study, study dose, chemotherapy backbone, age, and sex. Candidate markers were tested for association with hypertension using multiple logistic regression while correcting for these covariates. Markers that were nominally associated ($P \leq 0.05$) with bevacizumab-induced hypertension in white patients were also tested for association with progression-free survival (PFS), overall survival (OS), and best overall response according to Response Evaluation Criteria in Solid Tumors using Cox proportional hazards regression or multiple logistic regression, as appropriate, while adjusting for relevant covariates. To assess linkage disequilibrium, the Lewontin's D′ statistic was calculated using the Haploview software version 4.2 [43].

Results

Patient population

Overall, 1,631 patients in the six trials consented to participate in the genetic biomarker substudy. Of these, approximately equal numbers received bevacizumab with standard therapy ($n = 807$) or placebo with standard therapy ($n = 824$). The distribution of patients across the six trials and their baseline characteristics are summarized in Table 1. With the exception of AViTA and AVAGAST, all trials met their primary objective. Overall, no

Table 1 Summary of demographic and recruitment data

	AVAiL	AVOREN	AViTA	AVADO	NO16966	AVAGAST	All
Cancer type	NSCLC	Renal	Pancreatic	Breast	Colorectal	Gastric	
Recruitment period	Feb 2005–Aug 2006	Jun 2004–Oct 2005	Jul 2005–Sep 2006	Mar 2006–Apr 2007	Feb 2004–Feb 2005	Sep 2007–Dec 2008	
Patients participating in the clinical study (*n*)	1,043	649	607	736	1,400	774	5,209
Patients consenting to the genetic substudy (%)	119	108	160	348	610	286	1,631
Bevacizumab [*n* (%)]	83 (70)	59 (55)	79 (49)	238 (68)	210 (34)	138 (48)	807 (49)
Placebo [*n* (%)]	36 (30)	49 (45)	81 (51)	110 (32)	400 (66)	148 (52)	824 (51)
Male (%)	71	72	64	0	61	66	50
Mean age [years (SD)]	57.4 (9.9)	59.7 (10.6)	61.6 (9.6)	54.7 (10.8)	59.6 (11.5)	57.2 (11.3)	58.2 (11.2)
White (%)	92	99	95	96	85	63	86

NSCLC non-small cell lung cancer, *SD* standard deviation

Table 2 Summary of clinical outcomes in bevacizumab-treated arms and incidences of hypertension

	AVAiL[a]	AVOREN	AVITA	AVADO[a]	NO16966	AVAGAST	All
Cancer type	NSCLC	Renal	Pancreatic	Breast	Colorectal	Gastric	
Median OS in genetic substudy, months	13.9	33.2	7.0	28.7	20.9	9.3	17.1
Censored OS in the genetic substudy (%)	36	47	9	37	19	29	27
Median PFS in genetic substudy, months	6.5	13.6	4.9	8.4	8.8	5.5	7.9
Censored PFS in genetic substudy (%)	3	11	4	7	4	14	7
Hypertension in the bevacizumab arm [*n* (%)]	21 (25)	17 (29)	14 (18)	41 (17)	34 (16)	16 (12)	143 (18)
Hypertension in the placebo arm [*n* (%)]	4 (11)	5 (10)	7 (9)	13 (12)	15 (4)	7 (5)	51 (6)
Hypertension in both treatment arms [*n* (%)]	25 (21)	22 (20)	21 (13)	54 (16)	49 (8)	23 (8)	194 (12)

NSCLC non-small cell lung cancer, *OS* overall survival, *PFS* progression-free survival

[a] Bevacizumab 15 mg/kg arm only

substantial differences in terms of PFS or OS were noted between each genetic substudy and the corresponding clinical studies. Hypertension was reported in 143 (18 %) of 807 bevacizumab-treated patients and 51 (6 %) of 824 placebo-treated patients. Across individual studies, the incidences of hypertension (irrespective of treatment administered) ranged from 8 % in AVAGAST and NO16966 to 21 % in AVAiL (Table 2).

VEGF-A pathway SNPs and bevacizumab-induced hypertension

Of the 15 VEGF-A pathway genes, six SNPs were nominally ($P \leq 0.05$) associated with bevacizumab-induced hypertension in white patients (Table 3), but none of these surpassed the threshold for multiple testing ($P < 0.0002$). The strongest association was for rs1680695 in *EGLN3,* with an allelic odds ratio (OR) of 1.50 (95 % confidence interval [CI], 1.09–2.07; $P = 0.012$; Table 3; Fig. 1a). Specifically, 14 (20 %) of 70 GG carriers treated with bevacizumab developed hypertension versus 58 (21 %) of 281 TG carriers and 34 (12 %) of 276 TT carriers (Fig. 2a). In the placebo arm, 10, 7, and 6 %, respectively, developed hypertension.

The SNP with the second strongest association was rs2305949 in *KDR* (OR 0.62; 95 % CI 0.42–0.93; $P = 0.020$; Table 3; Fig. 1b). In patients treated with bevacizumab, the incidence of hypertension was highest in CC genotype carriers (81 [19 %] of 418 CC carriers vs. 29 [14 %] of 212 CT carriers and 3 [10 %] of 31 TT carriers; Fig. 2b). In the placebo arm, corresponding incidences were 5, 9 and 12 %, respectively. Intriguingly, this marker is in linkage disequilibrium with the non-synonymous variant rs2305948 (valine to isoleucine) occurring at position 297 of *KDR* ($D' = 1$), suggesting that this change may functionally affect VEGF-A pathway signaling.

Hypertension-specific SNPs and bevacizumab-induced hypertension

Of the selected markers outside the VEGF-A pathway, four SNPs were nominally ($P \leq 0.05$) associated with

Table 3 *VEGF-A* pathway SNPs with $P \leq 0.05$ for association with hypertension

Marker	Chr	Base pair[a]	MAF	HWE *P* value	*N*	OR	95 % CI	*P* value	Gene
rs1680695	14	34,408,083	0.35	0.64	627	1.50	1.09–2.07	0.012	*EGLN3*
rs2305949	4	55,980,456	0.21	0.26	661	0.62	0.42–0.93	0.020	*KDR*
rs4953340	2	46,548,064	0.36	0.52	612	1.44	1.05–1.96	0.023	*EPAS1*
rs2034327	2	46,549,040	0.49	0.82	447	0.68	0.47–0.98	0.037	*EPAS1*
rs111458691	13	29,069,942	0.03	0.51	656	0.12	0.02–0.91	0.041	*FLT1*
rs1130379	5	180,039,606	0.08	0.01	557	1.72	1.00–2.95	0.050	*FLT4*

Chr chromosome, *CI* confidence interval, *HWE* Hardy–Weinberg equilibrium, *MAF* minor allele frequency, *OR* odds ratio, *SNP* single nucleotide polymorphism

[a] Base-pair positions are calculated based on reference genome hg19

Table 4 Hypertension SNPs with $P \leq 0.05$ for association with bevacizumab-induced hypertension

Marker	Chr	Base pair[a]	MAF	HWE *P* value	*N*	OR	95 % CI	*P* value	Gene
rs4444903	4	110,834,110	0.42	0.46	683	1.57	1.17–2.11	0.0025	*EGF*
rs9992755	4	110,882,590	0.36	0.29	658	1.45	1.08–1.96	0.014	*EGF*
rs1937506	13	68,035,371	0.25	0.52	644	1.53	1.09–2.15	0.015	-[b]
rs11064560	12	943,953	0.33	0.04	689	1.41	1.04–1.92	0.028	*WNK1*

Chr chromosome, *CI* confidence interval, *HWE* Hardy–Weinberg equilibrium, *MAF* minor allele frequency, *OR* odds ratio, *SNP* single nucleotide polymorphism

[a] Base-pair positions are calculated based on reference genome hg19

[b] The rs1937506 SNP is located in a gene desert and its function is unknown. It is located 230 kbp upstream of *PCDH9*

bevacizumab-induced hypertension (Table 4), but none of these surpassed the threshold for multiple testing ($P < 0.0002$). The SNP showing the strongest association was rs4444903 in *EGF* (OR 1.57; 95 % CI 1.17–2.11; $P = 0.0025$; Fig. 1c). At the genotype level, the mutant G-allele of rs4444903 was associated with bevacizumab-induced hypertension. In bevacizumab-treated patients, hypertension was reported in 18 (8 %) of 232 AA carriers versus 76 (24 %) of 321 AG carriers and 21 (16 %) of 130 GG carriers (Fig. 2c). Corresponding values in placebo-treated patients were 5, 7, and 6 %, respectively. The rs4444903 is closely linked to rs2237051, which involves an amino acid change (methionine to isoleucine) at position 708 of *EGF*, suggesting that this change may functionally affect *EGF* activity and thereby contribute to hypertension. Remarkably, another marker in *EGF* (rs9992755), which is 48 kbp away from rs4444903, was also associated with hypertension ($P = 0.014$; Table 4). Both markers are in linkage disequilibrium with the rs2237051 non-synonymous change ($D' = 0.92$ for rs4444903; $D' = 0.87$ for rs9992755).

One additional SNP in *WNK1*, rs11064560, was associated with bevacizumab-induced hypertension ($P = 0.028$). The allelic OR was 1.41 (95 % CI 1.04–1.92; $P = 0.028$; Figs. 1d, 2d).

Effect of hypertension-associated SNPs on efficacy endpoints

Finally, we assessed whether SNPs that correlated significantly with bevacizumab-induced hypertension were also associated with treatment efficacy (best overall response, PFS, or OS). We identified one SNP in which the allele associated with hypertension also correlated with improved treatment response: the mutant G-allele of rs1680695 in *EGLN3* exhibited an improved best overall response compared with the wild-type T-allele (allelic OR 1.39; 95 % CI 1.08–1.80; $P = 0.011$). However, no significant effect of rs1680695 was observed for PFS or OS in white patients treated with bevacizumab (PFS: allelic OR 0.89 [95 % CI 0.79–1.00], $P = 0.055$; OS: allelic OR 0.90 [95 % CI 0.79–1.04], $P = 0.16$). None of the other nine SNPs associated with bevacizumab-induced hypertension at $P \leq 0.05$ was associated significantly with either PFS or OS in the meta-analysis of all six studies (data not shown).

Discussion

In this large pooled analysis, several genetic variants in the VEGF-A pathway or in other genes linked to

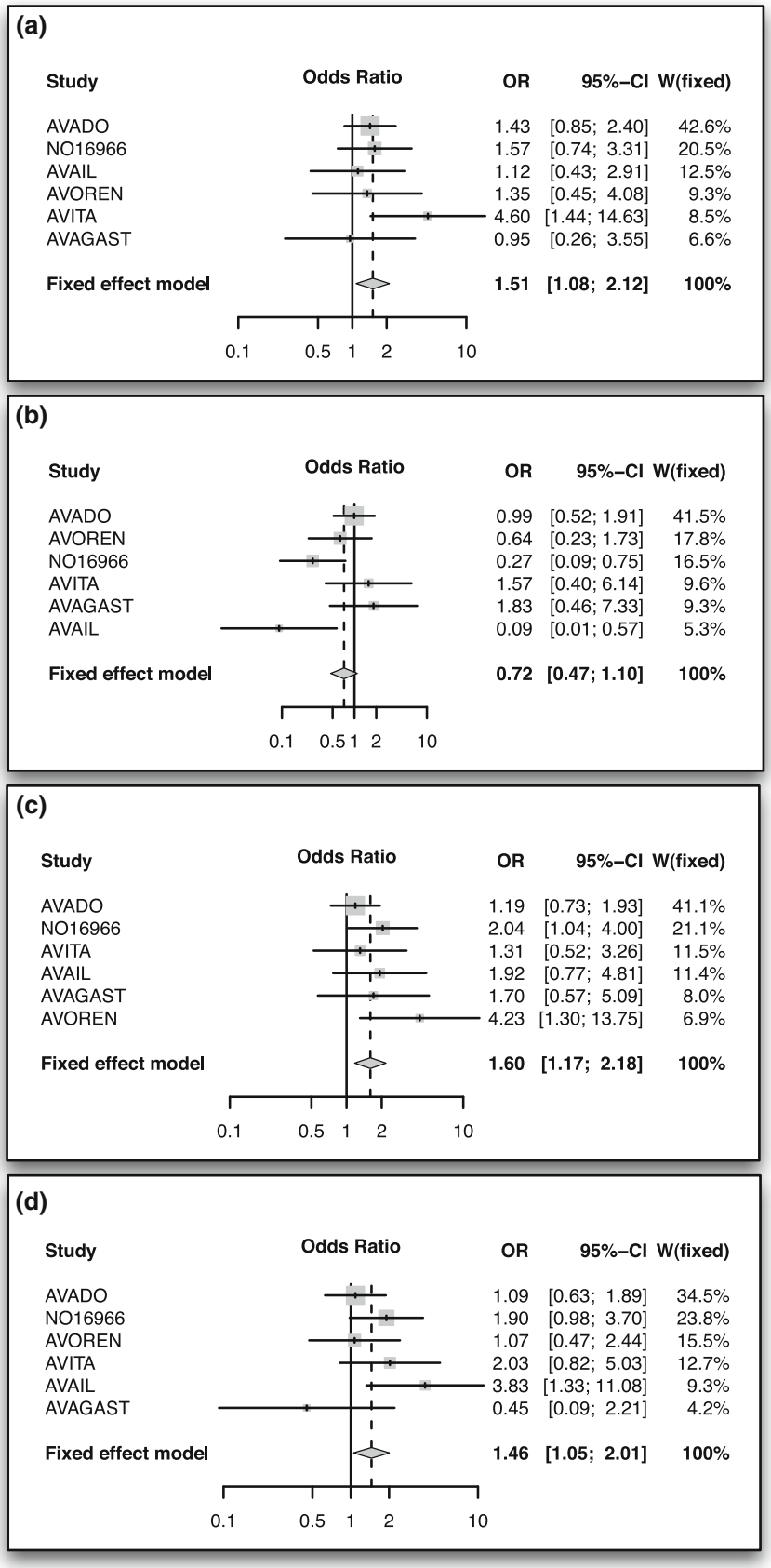

Fig. 1 Forest plot showing association of bevacizumab-induced hypertension with single nucleotide polymorphisms: **a** *EGLN3* rs1680695, **b** *KDR* rs2305949, **c** *EGF* rs4444903, and **d** *WNK1* rs11064560. Logarithmic ORs were calculated. Heterogeneity was assessed by I-square and tau-square values and a *P* value for heterogeneity was calculated. The *gray blocks* represent the ORs in the separate study populations. The *vertical dotted black line* represents the OR after meta-analysis. The *horizontal lines* are 95 % CIs. W(fixed) indicates the weight of each individual study. The ORs in these forest plots differ slightly from those in Tables 3 and 4 because ORs in the forest plot are calculated using a fixed-effects meta-analysis of summary statistics, whereas the ORs in Tables 3 and 4 are derived from a pooled meta-analysis of individual patient data (our main analysis approach). *Abbreviations*: *CI* confidence interval, *OR* odds ratio

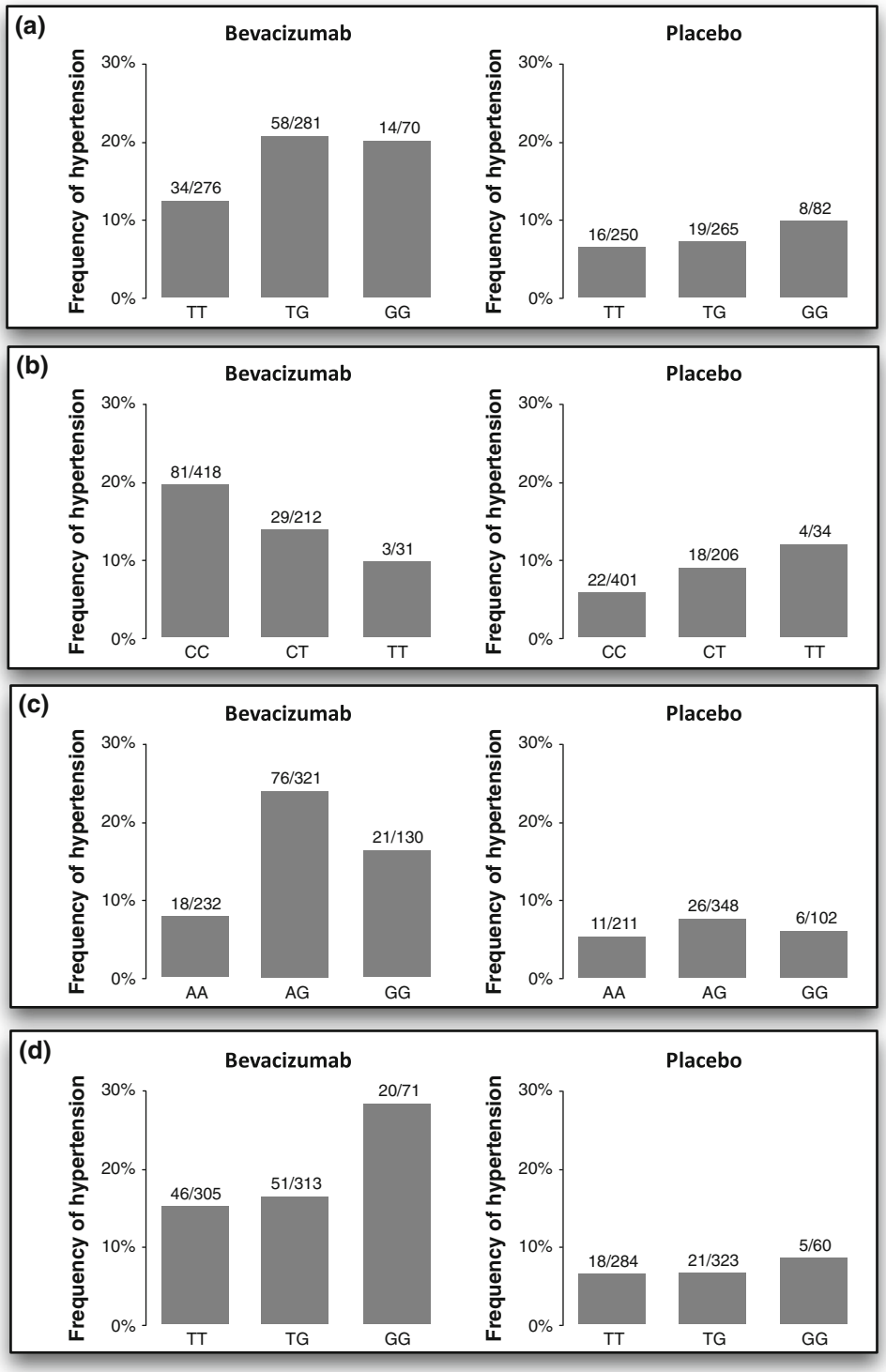

Fig. 2 *Bar* plot showing frequency of hypertension in bevacizumab- and placebo- treated patients stratified for single nucleotide polymorphisms: **a** *EGLN3* rs1680695, **b** *KDR* rs2305949, **c** *EGF* rs4444903, and **d** *WNK1* rs11064560

hypertension were associated with bevacizumab-induced hypertension. None of these associations was significant, however, after correction for multiple testing. Therefore these findings would require replication in additional studies and functional validation that affected genes are involved in hypertension before these variants can be considered as predictors of bevacizumab-induced hypertension.

For one of the variants, rs11064560 in *WNK1,* biological evidence supporting the observed association in a much smaller cohort is already available. Frey et al. observed a predictive association between two SNPs in *WNK1* (rs11064560 and rs2158501) and development of bevacizumab-induced hypertension in 28 patients with cancer [44]. The *WNK1* gene encodes a serine-threonine kinase that contributes to blood pressure homeostasis through regulation of the sodium chloride co-transporter in the distal convoluted tubule [45]. Mutations in *WNK1* also cause Gordon's syndrome, a rare Mendelian disorder characterized by hypertension and hyperkalemia [46]. In non-bevacizumab-treated populations, *WNK1* polymorphisms were associated with hypertension in a large family-based sample of white patients [47], and with ambulatory blood pressure and response to thiazide diuretics in African American and white cohorts [48]. Intriguingly, rs11064560 is in linkage disequilibrium with one of the SNPs identified in these studies, rs765250 ($D' = 1.00$). The rare allele of rs11064560, which correlated with bevacizumab-induced hypertension in the present study, is linked with the rs765250 G-allele that correlates with increased blood pressure. Thus the observed association with bevacizumab-induced hypertension is biologically plausible.

The rs2305949 SNP in *KDR* was associated with bevacizumab-induced hypertension in our study. The *KDR* gene contains a SNP in exon 7 (rs2305948; C/T) that results in a non-synonymous amino acid change at residue 297 in the third immunoglobulin-like domain. HEK293 cells transfected with a plasmid construct containing the rs2305948 TT genotype displayed a significantly lower VEGF-A binding affinity to KDR than did wild-type CC genotypes [49]. Likewise, we observed that human umbilical vein endothelial cells carrying rs2305948 TT genotypes proliferated significantly less than CC genotypes when exposed to recombinant *VEGF-A*, thereby corroborating these reports (unpublished observations). Of note, rs2305949 is in strong linkage with rs2305948 ($D' = 1$), suggesting that corresponding rs2305949 TT genotypes are also characterized by reduced VEGF-A/KDR interaction. This altered interaction might render the vasculature more susceptible to vascular rarefraction and concomitant hypertension.

We also observed that rs1680695 in *EGLN3* was associated with bevacizumab-induced hypertension and improved overall response in our meta-analysis of six trials. This indicates that carriers of the mutant G-allele may have an increased risk for hypertension but also a higher chance of a partial or complete response to bevacizumab. How *EGLN3* could contribute to bevacizumab-induced hypertension at the functional level is, however, not known. Another SNP involving an A to G mutation at position 61 of the 5′ untranslated region of *EGF* (rs4444903), which results in higher epidermal growth factor levels in GG-genotype carriers [50], was also associated with bevacizumab-induced hypertension in our study. This observation is noteworthy as the epidermal growth factor receptor (EGFR) may contribute to the development of hypertension by regulating vascular tone and renal sodium handling [51]. Synthetic EGFR inhibitors also reduce blood pressure in some experimental models of hypertension and have been suggested as a novel target for antihypertensive therapy [52].

The biological effects of the other SNPs that were associated with bevacizumab-induced hypertension at $P \leq 0.05$ are still unknown. Although our findings for each of these SNPs might be relevant, additional genetic and functional studies are needed to confirm their association, especially since blood pressure can be influenced by numerous environmental or clinical factors, thereby increasing the likelihood of detecting spurious associations. It should be noted, however, that to our knowledge the current study already represents by far the largest dataset of patients with bevacizumab-induced hypertension. Nevertheless, in the future, we anticipate investigating additional phase III clinical trials from the bevacizumab biomarker program—one of the largest biomarker programs in oncology—to increase further the number of patients assessed. Importantly, if the identified SNPs are replicated, they may be used to aid the selection of patients amenable to bevacizumab treatment. The potential clinical benefit of these SNPs is their ability to stratify patients into risk groups for developing hypertension before administration of the drug. Patients in the highest risk group could be monitored particularly closely for changes in blood pressure throughout treatment, allowing hypertension to be detected and treated without delay, or could possibly even allow selection of patients for prophylactic antihypertensive therapy.

In conclusion, this study represents a large genetic analysis of bevacizumab-induced hypertension using pooled datasets. Four markers, rs1680695 in *EGLN3*, rs2305949 in *VEGFR-2*, rs4444903 in *EGF,* and rs11064560 in *WNK1,* showed an association with hypertension. Additional studies are now warranted before considering the potential role for any of these SNPs in predicting the safety profile of bevacizumab.

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References

1. Maru D, Venook AP, Ellis LM (2013) Predictive biomarkers for bevacizumab: are we there yet? Clin Cancer Res 19(11):2824–2827. doi:10.1158/1078-0432.Ccr-12-3409
2. Hurwitz H, Fehrenbacher L, Novotny W et al (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350(23):2335–2342. doi:10.1056/NEJMoa032691
3. Saltz LB, Clarke S, Diaz-Rubio E et al (2008) Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 26(12):2013–2019. doi:10.1200/JCO.2007.14.9930
4. Bennouna J, Sastre J, Arnold D et al (2013) Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. Lancet Oncol 14(1):29–37. doi:10.1016/S1470-2045(12)70477-1
5. Sandler A, Gray R, Perry MC et al (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355(24):2542–2550. doi:10.1056/NEJMoa061884
6. Escudier B, Pluzanska A, Koralewski P et al (2007) Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 370(9605):2103–2111. doi:10.1016/S0140-6736(07)61904-7
7. Miller K, Wang ML, Gralow J et al (2007) Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 357(26):2666–2676. doi:10.1056/Nejmoa072113
8. Robert NJ, Dieras V, Glaspy J et al (2011) RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. J Clin Oncol 29(10):1252–1260. doi:10.1200/Jco.2010.28.0982
9. Burger RA, Brady MF, Bookman MA et al (2011) Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med 365(26):2473–2483. doi:10.1056/NEJMoa1104390
10. Perren TJ, Swart AM, Pfisterer J et al (2011) A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med 365(26):2484–2496. doi:10.1056/NEJMoa1103799
11. Aghajanian C, Blank SV, Goff BA et al (2012) OCEANS: A Randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol 30(17):2039–2045. doi:10.1200/Jco.2012.42.0505
12. Kreisl TN, Kim L, Moore K et al (2009) Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol 27(5):740–745. doi:10.1200/JCO.2008.16.3055
13. des Guetz G, Uzzan B, Chouahnia K, Morere JF (2011) Cardiovascular toxicity of anti-angiogenic drugs. Target Oncol 6(4):197–202. doi:10.1007/S11523-011-0204-7
14. Tang PA, Cohen SJ, Kollmannsberger C et al (2012) Phase II clinical and pharmacokinetic study of aflibercept in patients with previously treated metastatic colorectal cancer. Clin Cancer Res 18(21):6023–6031. doi:10.1158/1078-0432.Ccr-11-3252
15. Mross K, Frost A, Steinbild S et al (2012) A phase I dose-escalation study of regorafenib (BAY 73-4506), an inhibitor of oncogenic, angiogenic, and stromal kinases, in patients with advanced solid tumors. Clin Cancer Res 18(9):2658–2667. doi:10.1158/1078-0432.Ccr-11-1900
16. Ranpura V, Pulipati B, Chu D, Zhu X, Wu S (2010) Increased risk of high-grade hypertension with bevacizumab in cancer patients: a meta-analysis. AJH 23(5):460–468. doi:10.1038/ajh.2010.25
17. Budai B, Nagy T, Lang I, Hitre E (2013) The use of high dose d, l-leucovorin in first-line bevacizumab+ treatment of patients with metastatic colorectal cancer may enhance the antiangiogenic effect of bevacizumab. Angiogenesis 16(1):113–121. doi:10.1007/s10456-012-9303-z
18. Langenberg MH, van Herpen CM, De Bono J et al (2009) Effective strategies for management of hypertension after vascular endothelial growth factor signaling inhibition therapy: results from a phase II randomized, factorial, double-blind study of Cediranib in patients with advanced solid tumors. J Clin Oncol 27(36):6152–6159. doi:10.1200/JCO.2009.22.2273
19. Izzedine H, Ederhy S, Goldwasser F et al (2009) Management of hypertension in angiogenesis inhibitor-treated patients. Ann Oncol 20(5):807–815. doi:10.1093/Annonc/Mdn713
20. Ozcan C, Wong SJ, Hari P (2006) Reversible posterior leukoencephalopathy syndrome and bevacizumab. N Engl J Med 354(9):980–982; discussion 980–982. doi:10.1056/NEJMc052954
21. Allen JA, Adlakha A, Bergethon PR (2006) Reversible posterior leukoencephalopathy syndrome after bevacizumab/FOLFIRI regimen for metastatic colon cancer. Arch Neurol-Chicago 63(10):1475–1478. doi:10.1001/Archneur.63.10.1475
22. Sunshine SB, Dallabrida SM, Durand E et al (2012) Endostatin lowers blood pressure via nitric oxide and prevents hypertension associated with VEGF inhibition. Proc Natl Acad Sci U S A 109(28):11306–11311. doi:10.1073/pnas.1203275109
23. Kruzliak P, Kovacova G, Pechanova O (2013) Therapeutic potential of nitric oxide donors in the prevention and treatment of angiogenesis-inhibitor-induced hypertension. Angiogenesis 16(2):289–295. doi:10.1007/s10456-012-9327-4
24. Bouloumie A, Schini-Kerth VB, Busse R (1999) Vascular endothelial growth factor up-regulates nitric oxide synthase expression in endothelial cells. Cardiovasc Res 41(3):773–780. doi:10.1016/S0008-6363(98)00228-4
25. Henry TD, Annex BH, McKendall GR et al (2003) The VIVA trial: vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation 107(10):1359–1365
26. Facemire CS, Nixon AB, Griffiths R, Hurwitz H, Coffman TM (2009) Vascular endothelial growth factor receptor 2 controls blood pressure by regulating nitric oxide synthase expression. Hypertension 54(3):652–658. doi:10.1161/HYPERTENSIONAHA.109.129973
27. Kamba T, Tam BY, Hashizume H et al (2006) VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. Am J Physiol Heart Circ Physiol 290(2):H560–H576. doi:10.1152/ajpheart.00133.2005

28. Schneider BP, Wang M, Radovich M et al (2008) Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. J Clin Oncol 26(28):4672–4678. doi:10.1200/JCO.2008.16.1612
29. Van Cutsem E, Vervenne WL, Bennouna J et al (2009) Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. J Clin Oncol 27(13):2231–2237. doi:10.1200/JCO.2008.20.0238
30. Reck M, von Pawel J, Zatloukal P et al (2009) Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAiL. J Clin Oncol 27(8):1227–1234. doi:10.1200/Jco.2007.14.5466
31. Reck M, von Pawel J, Zatloukal P et al (2010) Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). Ann Oncol 21(9):1804–1809. doi:10.1093/annonc/mdq020
32. Escudier B, Bellmunt J, Negrier S et al (2010) Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): final analysis of overall survival. J Clin Oncol 28(13):2144–2150. doi:10.1200/JCO.2009.26.7849
33. Miles DW, Chan A, Dirix LY et al (2010) Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. J Clin Oncol 28(20):3239–3247. doi:10.1200/JCO.2008.21.6457
34. Ohtsu A, Shah MA, Van Cutsem E et al (2011) Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. J Clin Oncol 29(30):3968–3976. doi:10.1200/Jco.2011.36.2236
35. Lambrechts D, Lenz HJ, de Haas S, Carmeliet P, Scherer SJ (2013) Markers of response for the antiangiogenic agent bevacizumab. J Clin Oncol 31(9):1219–1230. doi:10.1200/Jco.2012.46.2762
36. Lambrechts D, Claes B, Delmar P et al (2012) VEGF pathway genetic variants as biomarkers of treatment outcome with bevacizumab: an analysis of data from the AViTA and AVOREN randomised trials. Lancet Oncol 13(7):724–733. doi:10.1016/S1470-2045(12)70231-0
37. Buysschaert I, Schmidt T, Roncal C, Carmeliet P, Lambrechts D (2008) Genetics, epigenetics and pharmaco-(epi)genomics in angiogenesis. J Cell Mol Med 12(6B):2533–2551. doi:10.1111/j.1582-4934.2008.00515.x
38. Wellcome Trust Case Control Consortium (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447(7145):661–678. doi:10.1038/nature05911
39. Conen D, Cheng S, Steiner LL, Buring JE, Ridker PM, Zee RY (2009) Association of 77 polymorphisms in 52 candidate genes with blood pressure progression and incident hypertension: the Women's Genome Health Study. J Hypertens 27(3):476–483
40. Smith NL, Hindorff LA, Heckbert SR et al (2007) Association of genetic variations with nonfatal venous thrombosis in postmenopausal women. JAMA 297(5):489–498. doi:10.1001/jama.297.5.489
41. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E et al (2004) 807 C/T Polymorphism of the glycoprotein Ia gene and pharmacogenetic modulation of platelet response to dual antiplatelet treatment. Blood Coagul Fibrinolysis 15(5):427–433
42. Reumers J, De Rijk P, Zhao H et al (2012) Optimized filtering reduces the error rate in detecting genomic variants by short-read sequencing. Nat Biotechnol 30(1):61–68. doi:10.1038/nbt.2053
43. Barret JC (2009) Haploview: Visualization and analysis of SNP genotype data. Cold Spring Harb Protoc 10:pdb.ip71. doi:10.1101/pdb.ip71
44. Frey MK, Olvera N, Bogomolniy F, et al. (2008) WNK1 haplotypes and bevacizumab-induced hypertension. J Clin Oncol 26(15 Suppl):11003
45. Huang CL, Kuo E (2007) Mechanisms of disease: WNK-ing at the mechanism of salt-sensitive hypertension. Nat Clin Pract Nephrol 3(11):623–630. doi:10.1038/ncpneph0638
46. Wilson FH, Disse-Nicodeme S, Choate KA et al (2001) Human hypertension caused by mutations in WNK kinases. Science 293(5532):1107–1112. doi:10.1126/science.1062844
47. Newhouse S, Farrall M, Wallace C et al (2009) Polymorphisms in the WNK1 gene are associated with blood pressure variation and urinary potassium excretion. PLoS ONE 4(4):e5003. doi:10.1371/journal.pone.0005003
48. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E (2005) WNK1 kinase polymorphism and blood pressure response to a thiazide diuretic. Hypertension 46(4):758–765. doi:10.1161/01.HYP.0000186240.81996.57
49. Wang Y, Zheng Y, Zhang W et al (2007) Polymorphisms of KDR gene are associated with coronary heart disease. J Am Coll Cardiol 50(8):760–767. doi:10.1016/j.jacc.2007.04.074
50. Tanabe KK, Lemoine A, Finkelstein DM et al (2008) Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA 299(1):53–60. doi:10.1001/jama.2007.65
51. Yoo BK, He P, Lee SJ, Yun CC (2011) Lysophosphatidic acid 5 receptor induces activation of Na(+)/H(+) exchanger 3 via apical epidermal growth factor receptor in intestinal epithelial cells. Am J Physiol Cell Physiol 301(5):C1008–C1016. doi:10.1152/ajpcell.00231.2011
52. Beltowski J, Lowicka E (2009) EGF receptor as a drug target in arterial hypertension. Mini Rev Med Chem 9(5):526–538