Cancer Chemotherapy and Pharmacology (2019) 84:1219–1227
https://doi.org/10.1007/s00280-019-03933-z

ORIGINAL ARTICLE

Endothelial nitric oxide synthase c.-813C>T predicts for proteinuria in metastatic breast cancer patients treated with bevacizumab-based chemotherapy

Stefania Crucitta[1] · Giuliana Restante[1] · Marzia Del Re[1] · Ilaria Bertolini[2] · Eleonora Bona[2] · Eleonora Rofi[1] · Lorenzo Fontanelli[1] · Giulia Gianfilippo[1] · Stefano Fogli[1] · Irene Stasi[2] · Matteo Ghilli[3] · Andrea Fontana[2] · Romano Danesi[1]

Received: 14 June 2019 / Accepted: 21 August 2019 / Published online: 16 September 2019

Abstract

Purpose To investigate the association between single nucleotide polymorphisms (SNPs) in endothelial nitric oxide synthase (*eNOS*) and interleukin-8 (*IL-8*) genes and risk of developing bevacizumab-related adverse events in metastatic breast cancer (mBC) patients.

Patients and methods mBC patients candidate to receive bevacizumab-based chemotherapy were enrolled in this pharmacogenetic study. *eNOS* c.-813C>T and c.894G>T, and *IL-8* c.-251A>T were analyzed by real time PCR on genomic DNA extracted from peripheral blood. Univariate analysis was performed to test the association between each SNP and treatment-related toxicities.

Results Seventy-six mBC patients were enrolled in the present study. Patients carrying the homozygous variant *eNOS* c.-813TT genotype showed a statistically significant occurrence of any grade proteinuria when compared to CT or CC genotypes ($p = 0.004$). No significant association of proteinuria with *IL-8* SNP or hypertension with selected *eNOS* and *IL-8* SNPs was found.

Conclusions These findings suggest an association between the *eNOS* c.-813C>T polymorphism and the development of proteinuria in mBC patients receiving a bevacizumab-based chemotherapy.

Keywords eNOS · Adverse events · Polymorphisms · Breast cancer · Bevacizumab · Proteinuria · Pharmacogenetics

Introduction

The monoclonal antibody bevacizumab (BEV) inhibits the binding between the vascular endothelial growth factor (*VEGF*) and its receptor, which signalling is a key mediator of tumor angiogenesis and promotes survival of tumor vessels [1]. However, despite the widespread use of bevacizumab, predictive biomarkers of efficacy and toxicity have not yet been identified in cancer patients treated with bevacizumab-based chemotherapy [2]. The adverse-event and mechanism of action profiles of bevacizumab make it a suitable drug for combination with chemotherapeutic agents, such as paclitaxel, for the treatment of metastatic breast cancer (mBC) patients [3, 4]. Miller et al. demonstrated that BEV increased progression-free survival (PFS) when combined with paclitaxel as first-line treatment in mBC [5]. As a result of down-regulation of cellular signalling pathways involved in the microvasculature growth and maintenance, adverse events (AEs) may occur, including hypertension and proteinuria [6, 7]. The exposure to anti-VEGF determines an increase in the systemic blood pressure and may cause endothelial dysfunction with simultaneous disruption of the filtration barrier in glomerular capillaries [8, 9]. The endothelial nitric oxide synthase (eNOS) is the major enzyme involved in the VEGF receptor

Stefania Crucitta and Giuliana Restante equally contributed to this work.

✉ Marzia Del Re
marzia.delre@gmail.com

[1] Unit of Clinical Pharmacology and Pharmacogenetics, Department of Clinical and Experimental Medicine, University of Pisa, 55, Via Roma, 56126 Pisa, Italy

[2] Unit of Medical Oncology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

[3] Unit of Breast Surgery, Breast Cancer Centre, University Hospital of Pisa, Pisa, Italy

2 (VEGFR-2) pathway [10] and, together with its downstream effector nitric oxide (NO), plays an important role in the maintenance of blood vessel integrity and normal renal function [11, 12]. In addition, interleukin-8 (IL-8), a key mediator of VEGF-independent pathway, induces angiogenesis increasing the endothelial permeability [13].

There is accumulating evidence that two *eNOS* single nucleotide polymorphisms (SNPs) (c.-813C>T, rs2070744 and c.894G>T, rs1799983) are associated with modulation of eNOS activity and formation of NO [14–18]. Previously published reports in bevacizumab-treated patient suggested a relationship between vaso-modulatory factors, BEV, and genetic variants occurring as SNPs in the *eNOS* gene. In particular, the *eNOS* c.894G>T polymorphism seems to be associated with a higher risk of developing grade 3–4 hypertension and proteinuria in metastatic colorectal cancer patients treated with bevacizumab [19, 20]. Moreover, the A variant of *IL-8* c.-251T>A polymorphism has been associated with increased IL-8 production and changes in the promoter transcriptional activity, and it has been previously associated with bevacizumab-related progression free survival (PFS) [20, 21]. However, no previous studies investigated the associations between hypertension and proteinuria and *eNOS* and *IL-8* SNPs in patients treated with bevacizumab.

In the present study, *eNOS* and *IL-8* polymorphisms were selected on the basis of the published studies on their involvement in cardiovascular and renal toxicity, and their role in a direct or indirect modulation of angiogenesis [20, 22, 23] and the objective was to investigate the correlation between *eNOS* c.-813C>T, c.894C>T, and *IL-8* c.-251A>T polymorphisms with AEs and outcome in mBC patients treated with bevacizumab-based chemotherapy.

Patients and methods

Patient selection

The study is a mono-institutional pharmacogenetic study that enrolled breast cancer patients receiving bevacizumab and paclitaxel as first-line treatment for advanced disease. Inclusion criteria were: history of advanced (loco-regionally recurrent or metastatic) breast cancer, histologically confirmed human epidermal growth factor receptor 2 (HER2) negative disease, age > 18 years old, willingness to undergo blood sample collection for biological assessments as scheduled in the protocol. The study was approved by the Ethics Committee of Pisa University Hospital and conducted in accordance with the principles of the Declaration of Helsinki. All patients gave their signed informed consent before blood collection and data analysis.

Blood sample collection, DNA isolation, and SNP genotyping

Blood samples were collected in tubes containing EDTA and stored at − 80 °C until analysis. Genomic DNA was extracted from 200 µl of whole blood using QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The analysis of *eNOS* c.-813C>T, c.894C>T, and *IL-8* c.-251A>T was conducted on a 7900 HT Real Time PCR (Applied Biosystem, Foster City, CA, USA) according to the manufacturer's standard protocol. In Table 1 are reported name, reference number, molecular effect, and clinical significance of the selected polymorphisms.

Statistical analysis

Categorical data were described by absolute and relative frequencies, whereas quantitative data were reported as mean and standard deviation. The association between each gene polymorphism with AEs has been evaluated by Chi-square test, according to the dominant and the recessive models of inheritance. Levene's test and Mann–Whitney analysis were used to evaluate the different grade of AEs and SNPs. PFS and overall survival (OS) were defined as the time from treatment start to progression disease or death, respectively. The Kaplan–Meier method was used to create PFS curves and log-rank test was used to evaluate the differences between curves. Hazard ratio was calculated to compare cumulative risks. Univariate analysis was performed to evaluate the correlation between clinical parameters

Table 1 Selected *eNOS* and *IL-8* gene polymorphisms and their molecular effects

Gene symbol	Complementary DNA variant	rs number	Molecular effect	Biological significance	References
eNOS	c.-813C>T	2070744	Reduced promoter activity of C allele	Modulation of NO formation and vasodilation	[24, 36]
eNOS	c.894G>T	1799983	Reduced enzyme activity of T allele	Modulation of NO formation and vasodilation	[17, 24]
IL-8	c.-251A>T	4073	Reduced transcriptional activity of T allele	Modulation of IL-8 production and inflammation	[21]

rs reference SNPs identification number, *SNPs* single nucleotide polymorphisms, *N* number of patients, *IL-8* interleukin-8, *eNOS* endothelial nitric oxide synthase, *NO* nitric oxide

and AEs. Statistical significance was defined by a p value of 0.05. Analysis was performed by MedCalc version 14.8.1. Toxicity grade is reported following the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

Results

The study included a total of 76 HER2-negative mBC patients treated from 2008 to 2013 at our institution with bevacizumab-based chemotherapy for advanced disease, as per clinical practice. Bevacizumab maintenance, alone or with hormonal therapy in case of hormone receptor positive disease, was allowed after paclitaxel discontinuation without disease progression. Sixty-four patients had hormone receptor (estrogen and/or progesterone) positive tumor, while 12 patients had a triple negative breast cancer. Visceral involvement was present in 60 patients, and 16 patients had non-visceral disease. Chemotherapy for early stage disease was administered to 52 patients; in 16 cases, a taxane-containing regimen was used. Ten patients already had a cardiovascular comorbidity before the start of the antiangiogenic therapy, 8 patients developed hypertension (all of them were therapy-controlled), and 2 reported arrhythmias. Main characteristics of patients are reported in Table 2.

Thirty-seven (49%) patients had a partial response (PR) and 5 (6.5%) had a complete response (CR), while 5 (6.5%) subjects had minor response (MR), 19 (25%) patients had stable disease (SD) and 7 (9%) had progressive disease (PD). In 3 patients (4%) tumor response cannot be assessed. The median PFS and OS were 10.9 months (95% CI 9.7–13.8 months) and 38.2 months (95% CI 31–45.9 months), respectively. Fifty-five patients (72%) developed hypertension and 35 patients (46%) developed proteinuria. In detail, 39 (71%) and 12 (34%) patients developed grade 2–3 of hypertension and proteinuria, respectively. None of the patients experienced grade 4 hypertension or proteinuria.

The calculation of Hardy–Weinberg equilibrium was performed; nevertheless, polymorphisms were found not to be in equilibrium. The distribution of SNPs is presented in Tables 3 and 4, which reports the frequency of patients who developed toxicities, stratified as per genotype.

Stratifying patients by genotypes and AEs, subjects carrying the homozygous mutant *eNOS* c.-813TT genotype showed a statistically significant incidence of any grade proteinuria when compared to CC or CT genotypes ($p = 0.004$) (Table 4, Fig. 1). Univariate analysis was performed considering clinical parameters and proteinuria development, such as age, site of disease, and number of metastatic sites. None of them was significantly associated with any grade proteinuria, confirming the role of the *eNOS* c.-813TT genotype as independent predictive biomarker for the risk of

Table 2 Characteristics of patients

Clinical parameters	$N = 76$	%
Years of age—median (range)	52.5 (34–74)	
Hormonal status		
ER+/PgR±	64	84
ER−/PgR−	12	16
Previous (neo) adjuvant chemotherapy		
None	24	32
Anthracycline	24	32
Taxane	16	21
Other	12	16
Previous hormonal therapy[a]		
Adjuvant setting	47	62
Metastatic setting	24	32
Location of disease		
Breast	13	17
Visceral	60	79
Bone-only	3	4
Stage at initial diagnosis		
Stage I	12	16
Stage II	25	33
Stage III	21	28
Stage IV	17	22
Unknown	1	1.3
Cardiovascular comorbidities		
Hypertension	8	11
Other	2	3

[a]Previous hormonal therapy includes ER+/PgR± patients only, ten of whom received hormonal therapy both in adjuvant and metastatic setting

Table 3 Frequency of *eNOS* and *IL-8* SNPs in patients treated with bevacizumab-based chemotherapy

SNP	Genotype	*N*	%
eNOS c.-813C>T	TT	19	25
	TC	28	36.9
	CC	29	38.1
eNOS c.894G>T	GG	31	40.8
	GT	40	52.6
	TT	5	6.6
IL-8 c.-251 A>T[a]	TT	17	24
	TA	43	60.5
	AA	11	15.5

SNPs single nucleotide polymorphisms, *N* number of patients, *IL-8* interleukin-8, *eNOS* endothelial nitric oxide synthase

[a]Five samples were not available for the molecular analysis of *IL-8*

Table 4 Associations of bevacizumab-related specific toxicities and *eNOS*/*IL-8* SNPs[a]

SNPs	Hypertension			Proteinuria		
	G 0	G 1–3	*p* value	G 0	G 1–3	*p* value
eNOS c.-813C>T						
TT	4 (22.2%)	14 (77.8%)	0.78	4 (22.2%)	14 (77.8%)	0.004
CT+CC	14 (25.5%)	41 (74.5%)		34 (61.8%)	21 (38.2%)	
eNOS c.894 G>T						
TT	0 (0%)	5 (100%)	0.92	3 (60%)	2 (40%)	0.71
GT+GG	18 (26.5%)	50 (73.5%)		35 (51.5%)	33 (48.5%)	
IL-8 c.-251A>T						
TT	5 (31.3%)	11 (68.7%)	0.41	9 (56.2%)	7 (43.8%)	0.67
TA+AA	11 (21.2%)	41 (78.8%)		26 (50%)	26 (50%)	

SNPs single nucleotide polymorphisms, *G 0* grade zero toxicity—no toxicity, *G 1–3* grade 1–3 toxicity, *rs* reference SNP identification number, *eNOS* endothelial nitric oxide synthase, *IL-8* interleukin-8, [a]in three patients the clinical information on hypertension or proteinuria is not available

proteinuria development in mBC patients treated with bevacizumab (Table 5). Figure 2 shows the possible mechanisms underlying our findings.

No significant correlation of *eNOS* c.894G>T and *IL-8* c.-352A>T and proteinuria was found (Table 4, Fig. 1). Moreover, *eNOS* and *IL-8* SNPs failed to show any significant correlation with hypertension (Table 4, Fig. 1) or PFS and OS ($p > 0.05$, Fig. 3).

Discussion

The present study analysed selected polymorphisms in the *eNOS* and *IL-8* and their associations with AEs in mBC patients receiving bevacizumab-based chemotherapy, demonstrating a significant correlation between the development of any grade proteinuria and the c.-813TT genotype. Several studies investigated the role *eNOS* and *IL-8* polymorphisms as biomarkers owing to their role in *VEGF*-dependent and independent pathways [6, 7, 20, 21, 24, 25]. In particular, there is accumulating evidence that two *eNOS* SNPs (c.-813C>T and c.894G>T) are associated with decreased *eNOS* activity and formation of NO [14–17, 26–28]. A research investigating the role of *eNOS*, *VEGF*, and endothelin-1 (*ET-1*) polymorphisms in 255 metastatic renal cancer patients treated with the anti-VEGFR sunitinib showed that the presence of a particular haplotype in *VEGFA* and the *eNOS* c.-813C>T SNP were independent predictors for the risk of developing grade 3 hypertension ($p = 0.031$ and $p = 0.045$, respectively) [29]. These results highlighted the correlation between germline SNPs and the development of hypertension in patients treated with anti-VEGF drugs [30–33]. However, another study in patients affected by hepatocarcinoma failed to show a statistically significant association between *eNOS* polymorphisms and hypertension in patients treated with

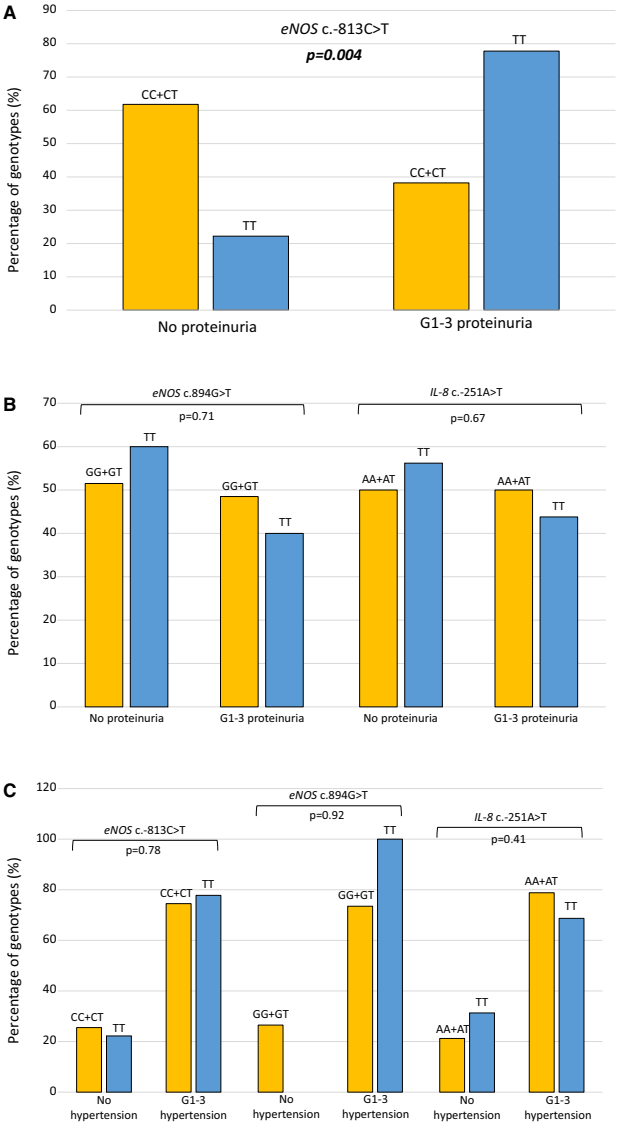

Fig. 1 Incidence of *eNOS* c.-813C>T (**a**), *eNOS* c.894G>T and *IL-8* c.-251A>T genotypes in grade 0 vs. 1–3 proteinuria (**b**) and *eNOS* c.-813C>T, c.894G>T, and *IL-8* c.-251A>T genotypes in grade 0 vs. 1–3 hypertension (**c**)

Table 5 Univariate analysis clinical variables influencing proteinuria

Variable	OR (95% CI)	*p* value
Age (< 49 vs. ≥ 49)	0.4583 (0.1762–1.1921)	0.1097
Disease sites (visceral vs. no visceral)	0.7621 (0.2442–2.3783)	0.6399
Number of metastatic sites (< 3 vs. ≥ 3)	1.9636 (0.7545–5.1103)	0.1667

sorafenib, but an association between hypertension and a better clinical outcome was observed [34]. In particular, the study showed that patients with increased systolic or diastolic blood pressure after 15 days of treatment with the anti-VEGFR sorafenib had better PFS and OS ($p = 0.005$ and $p = 0.027$, respectively) [34]. In our study, we showed for the first time a statistically significant association of proteinuria and the *eNOS* c.-813TT genotype compared to patients carrying the CC or CT genotype ($p = 0.004$). The development of proteinuria is probably due to a dysfunction of glomerular endothelial cells [35], due to the protective role of *eNOS* against renal damage [30]. The *eNOS* c.-813C>T polymorphism is associated with reduced transcriptional activity of the C allele [36, 37]. Conversely, subjects' carriers of the *eNOS* c.-813T allele have a normal or enhanced eNOS expression and NO formation [24]. *eNOS* plays an important role as mediator of renal endothelial functions as its expression is up-regulated in the afferent and glomerular endothelium in association with an increase in diameter of afferent arterioles, glomerular volume, and filtration rate [38, 39]. Moreover, genetic association studies showed that the *eNOS* c.-813C>T polymorphism may be implicated in the development of nephropathy [40, 41] and this finding may explain the increased occurrence of proteinuria in patients' carrier of the *eNOS* c.-813TT genotype as showed in our study. Nevertheless, these results need to be further investigated in studies evaluating additional gene regulation mechanisms. Recently, Di Salvatore et al. found an association between bevacizumab-related adverse events and survival and *eNOS* and *IL-8* SNPs [19, 20]. In 120 metastatic colorectal cancer patients treated with FOLFOX regimen plus bevacizumab, the *eNOS* c.894TT genotype was associated with higher grades of hypertension and proteinuria

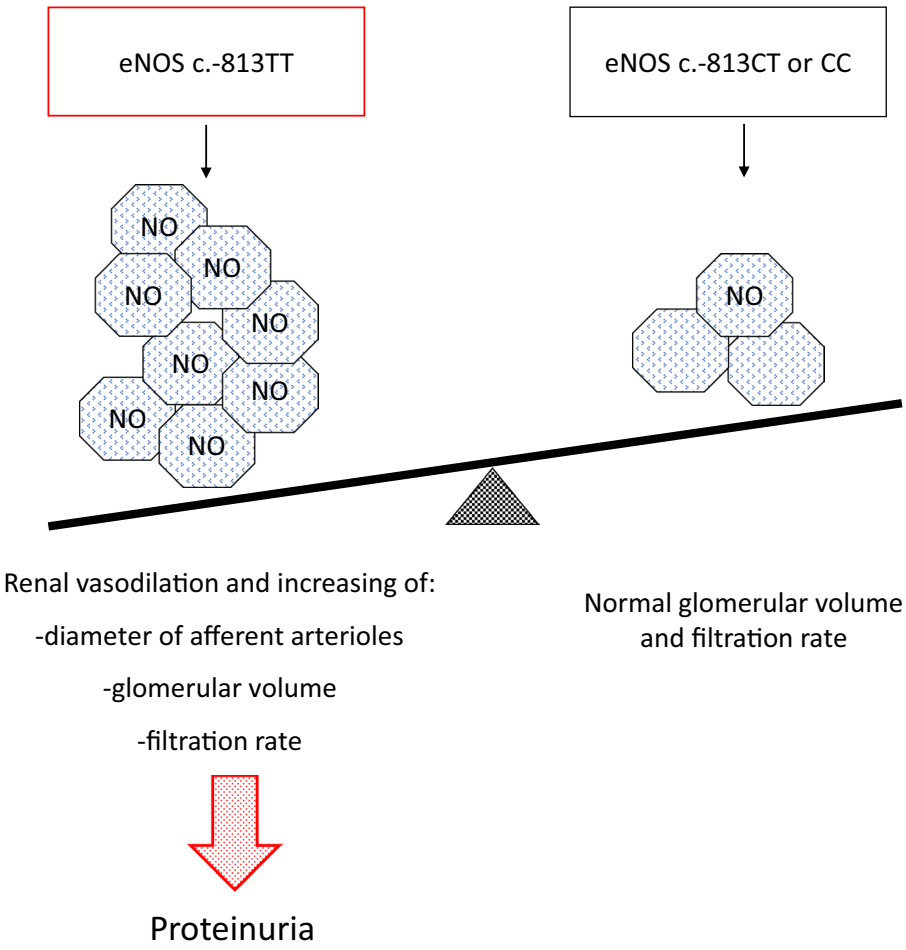

Fig. 2 Pre-transcriptional effects of eNOS c.-813C>T polymorphisms and association with proteinuria. The c.-813T variant in the promoter region of the eNOS gene is associated with higher transcriptional activity and NO production than c.-813 C variant. eNOS c.-813 TT genotype may be associated with renal capillaries vasodilation and a consequent increase of the diameter of afferent arterioles, glomerular volume, filtration rate, and proteinuria

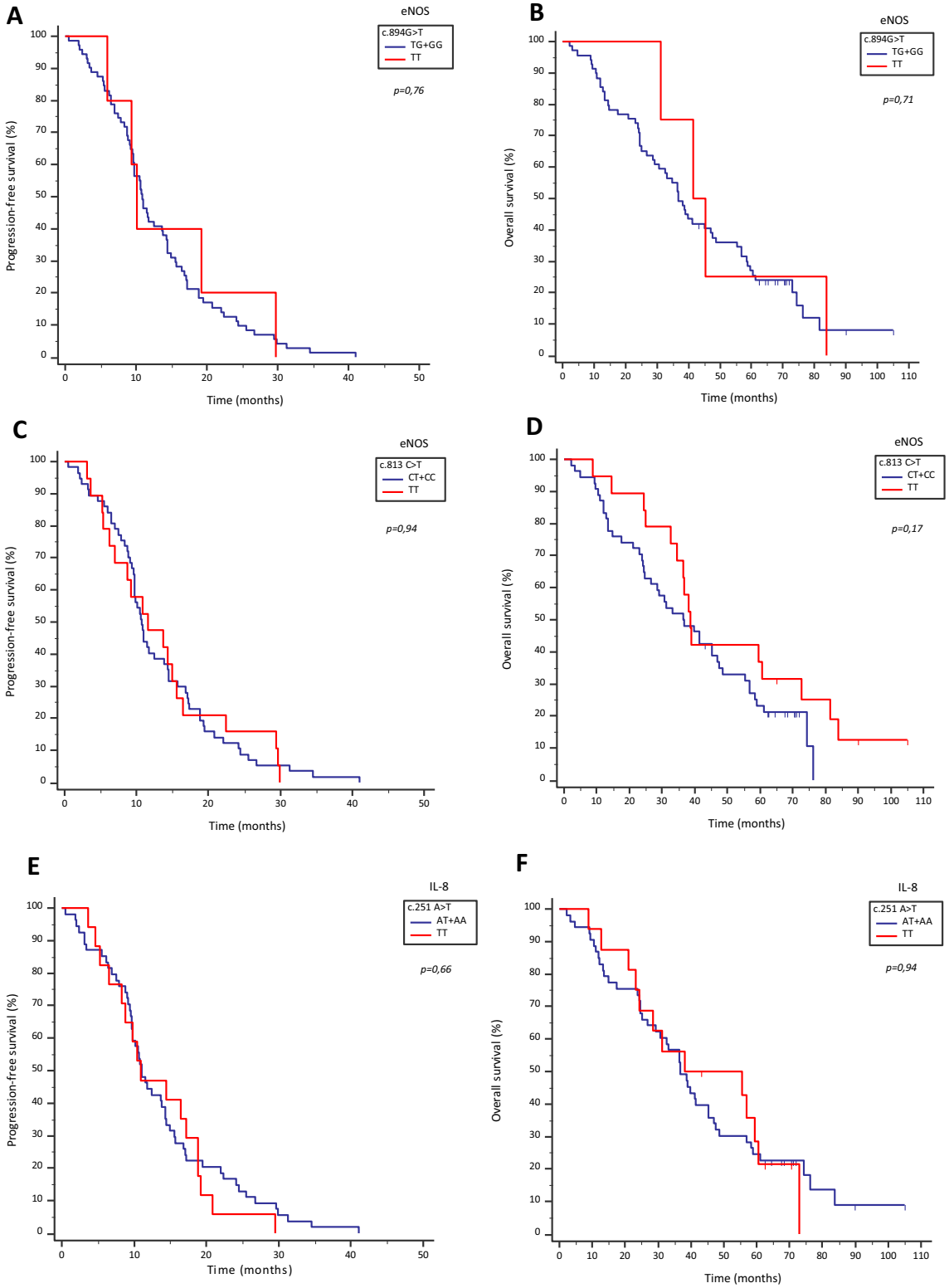

Fig. 3 Progression-free survival (PFS) and overall survival (OS) in patients with *eNOS* genotype c.894TT vs. GT+GG (**a**, **b**) and c.-813TT vs. CT+CC (**c**, **d**) and *IL-8* c.251TT vs. AT+AA (**e**, **f**)

($p = 0.0002$). Moreover, patients carrying c.894TT genotype had a better PFS compared to patients carrying c.894 GG/GT genotype ($p = 0.049$) [19, 20]. Even if the role of the *eNOS* SNP was confirmed as predictive biomarker of AEs, no significant correlation between *eNOS* c.813C>T and c.894G>T SNPs and clinical outcomes (PFS and OS) was found in our study, probably because of the different type of disease and treatments. On the contrary, *IL-8* c.-251T>A and *eNOS* c.-894G>T SNPs were significantly associated with treatment outcome of bevacizumab in 31 metastatic breast cancer patients [19]. Concerning the *IL-8* gene, the A variant of the c.-251A>T polymorphism is associated with increased IL-8 production and activation of transcriptional activity [21]. In fact, high levels of IL-8 in tumour vessels stimulate the production of the vasodilators NO and prostacyclin, and may promote the endothelial dysfunction and the deregulation of blood pressure [42]. Patients carrying this SNP may have an unfavourable genetic profile [21] and it was demonstrated that IL-8 could be a molecular predictor of response to bevacizumab-based chemotherapy [19]. Moreover, a published study showed the involvement of IL-8 as a risk factor of severe pregnancy-related disorder (i.e., hypertension, proteinuria, and multiple organ damage) and it was observed a significantly higher frequency *IL-8* c.-251A allele in cases compared to the control group [42]. Nevertheless, in our study, no significant association between *IL-8* SNP, bevacizumab-related toxicities, and PFS/OS was observed. Although our results are stimulating and hypothesis-generating, our study is limited by its retrospective nature, the small sample size, and the limited number of SNPs evaluated. However, it would be interesting to evaluate the possible correlation between *eNOS* c.-813C>T polymorphism and proteinuria in a larger validation cohort. Moreover, the current approach to correlate bevacizumab-related AEs to a single SNP may be replaced by a network-based approach to evaluate the interaction between different multiple SNPs (i.e., haplotype) and AEs. Further studies are needed to understand the influence of the *eNOS* variants in relation to the inhibition of the VEGF pathway.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Rajabi M, Mousa SA (2017) The role of angiogenesis in cancer treatment. Biomedicines 5:2. https://doi.org/10.3390/biomedicines5020034
2. Giuliano S, Pages G (2013) Mechanisms of resistance to anti-angiogenesis therapies. Biochimie 95(6):1110–1119. https://doi.org/10.1016/j.biochi.2013.03.002
3. Redondo A, Ramos Vazquez M, Manso L, Gil Gil MJ, Garau Llinas I, Garcia-Garre E, Rodriguez CA, Chacon JI, Lopez-Vivanco G (2018) Long-term response to first-line bevacizumab-based therapy in patients with metastatic breast cancer: results of the observational "LORENA" study. Onco Targets Ther 11:5845–5852. https://doi.org/10.2147/OTT.S170303
4. Raouf S, Bertelli G, Ograbek A, Field P, Tran I (2019) Real-world use of bevacizumab in metastatic colorectal, metastatic breast, advanced ovarian and cervical cancer: a systematic literature review. Future Oncol 15(5):543–561. https://doi.org/10.2217/fon-2018-0480
5. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, Davidson NE (2007) Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 357(26):2666–2676. https://doi.org/10.1056/NEJMoa072113
6. 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. Am J Hypertens 23(5):460–468. https://doi.org/10.1038/ajh.2010.25
7. Wu S, Kim C, Baer L, Zhu X (2010) Bevacizumab increases risk for severe proteinuria in cancer patients. J Am Soc Nephrol 21(8):1381–1389. https://doi.org/10.1681/ASN.2010020167
8. Schmidinger M (2013) Understanding and managing toxicities of vascular endothelial growth factor (VEGF) inhibitors. EJC Suppl 11(2):172–191. https://doi.org/10.1016/j.ejcsup.2013.07.016
9. Hayman SR, Leung N, Grande JP, Garovic VD (2012) VEGF inhibition, hypertension, and renal toxicity. Curr Oncol Rep 14(4):285–294. https://doi.org/10.1007/s11912-012-0242-z
10. Bachmann S, Bosse HM, Mundel P (1995) Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. Am J Physiol 268(5 Pt 2):F885–F898. https://doi.org/10.1152/ajprenal.1995.268.5.F885
11. Albrecht EW, Stegeman CA, Heeringa P, Henning RH, van Goor H (2003) Protective role of endothelial nitric oxide synthase. J Pathol 199(1):8–17. https://doi.org/10.1002/path.1250
12. Li M, Kroetz DL (2018) Bevacizumab-induced hypertension: clinical presentation and molecular understanding. Pharmacol Ther 182:152–160. https://doi.org/10.1016/j.pharmthera.2017.08.012
13. Petreaca ML, Yao M, Liu Y, Defea K, Martins-Green M (2007) Transactivation of vascular endothelial growth factor receptor-2 by interleukin-8 (IL-8/CXCL8) is required for IL-8/CXCL8-induced endothelial permeability. Mol Biol Cell 18(12):5014–5023. https://doi.org/10.1091/mbc.e07-01-0004
14. Tanus-Santos JE, Desai M, Deak LR, Pezzullo JC, Abernethy DR, Flockhart DA, Freedman JE (2002) Effects of endothelial nitric oxide synthase gene polymorphisms on platelet function, nitric oxide release, and interactions with estradiol. Pharmacogenetics 12(5):407–413
15. Doshi AA, Ziolo MT, Wang H, Burke E, Lesinski A, Binkley P (2010) A promoter polymorphism of the endothelial nitric oxide synthase gene is associated with reduced mRNA and protein expression in failing human myocardium. J Card Fail 16(4):314–319. https://doi.org/10.1016/j.cardfail.2009.12.013
16. Metzger IF, Sertorio JT, Tanus-Santos JE (2007) Modulation of nitric oxide formation by endothelial nitric oxide synthase gene haplotypes. Free Radic Biol Med 43(6):987–992. https://doi.org/10.1016/j.freeradbiomed.2007.06.012
17. Godfrey V, Chan SL, Cassidy A, Butler R, Choy A, Fardon T, Struthers A, Lang C (2007) The functional consequence of the Glu298Asp polymorphism of the endothelial nitric oxide synthase gene in young healthy volunteers. Cardiovasc Drug Rev

25(3):280–288. https://doi.org/10.1111/j.1527-3466.2007.00017.x
18. Veldman BA, Spiering W, Doevendans PA, Vervoort G, Kroon AA, de Leeuw PW, Smits P (2002) The Glu298Asp polymorphism of the NOS 3 gene as a determinant of the baseline production of nitric oxide. J Hypertens 20(10):2023–2027
19. Di Salvatore M, Lo Giudice L, Rossi E, Santonocito C, Nazzicone G, Rodriquenz MG, Cappuccio S, Inno A, Fuso P, Orlandi A, Strippoli A, Capoluongo E, Astone A, Cassano A, Barone C (2016) Association of IL-8 and eNOS polymorphisms with clinical outcomes in bevacizumab-treated breast cancer patients: an exploratory analysis. Clin Transl Oncol 18(1):40–46. https://doi.org/10.1007/s12094-015-1334-7
20. Di Salvatore M, Pietrantonio F, Orlandi A, Del Re M, Berenato R, Rossi E, Caporale M, Guarino D, Martinetti A, Basso M, Mennitto R, Santonocito C, Mennitto A, Schinzari G, Bossi I, Capoluongo E, Danesi R, de Braud F, Barone C (2017) IL-8 and eNOS polymorphisms predict bevacizumab-based first line treatment outcomes in RAS mutant metastatic colorectal cancer patients. Oncotarget 8(10):16887–16898. https://doi.org/10.18632/oncotarget.14810
21. Schultheis AM, Lurje G, Rhodes KE, Zhang W, Yang D, Garcia AA, Morgan R, Gandara D, Scudder S, Oza A, Hirte H, Fleming G, Roman L, Lenz HJ (2008) Polymorphisms and clinical outcome in recurrent ovarian cancer treated with cyclophosphamide and bevacizumab. Clin Cancer Res 14(22):7554–7563. https://doi.org/10.1158/1078-0432.CCR-08-0351
22. Ulivi P, Scarpi E, Passardi A, Marisi G, Calistri D, Zoli W, Del Re M, Frassineti GL, Tassinari D, Tamberi S, Vertogen B, Amadori D (2015) eNOS polymorphisms as predictors of efficacy of bevacizumab-based chemotherapy in metastatic colorectal cancer: data from a randomized clinical trial. J Transl Med 13:258. https://doi.org/10.1186/s12967-015-0619-5
23. Allegrini G, Coltelli L, Orlandi P, Fontana A, Camerini A, Ferro A, Cazzaniga M, Casadei V, Lucchesi S, Bona E, Di Lieto M, Pazzagli I, Villa F, Amoroso D, Scalese M, Arrighi G, Molinaro S, Fioravanti A, Finale C, Triolo R, Di Desidero T, Donati S, Marcucci L, Goletti O, Del Re M, Salvadori B, Ferrarini I, Danesi R, Falcone A, Bocci G (2014) Pharmacogenetic interaction analysis of VEGFR-2 and IL-8 polymorphisms in advanced breast cancer patients treated with paclitaxel and bevacizumab. Pharmacogenomics 15(16):1985–1999. https://doi.org/10.2217/pgs.14.140
24. Oliveira-Paula GH, Lacchini R, Tanus-Santos JE (2017) Clinical and pharmacogenetic impact of endothelial nitric oxide synthase polymorphisms on cardiovascular diseases. Nitric Oxide 63:39–51. https://doi.org/10.1016/j.niox.2016.08.004
25. Lafayette RA, McCall B, Li N, Chu L, Werner P, Das A, Glassock R (2014) Incidence and relevance of proteinuria in bevacizumab-treated patients: pooled analysis from randomized controlled trials. Am J Nephrol 40(1):75–83. https://doi.org/10.1159/000365156
26. Wang XL, Wang J (2000) Endothelial nitric oxide synthase gene sequence variations and vascular disease. Mol Genet Metab 70(4):241–251. https://doi.org/10.1006/mgme.2000.3033
27. Tesauro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J (2000) Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. Proc Natl Acad Sci USA 97(6):2832–2835. https://doi.org/10.1073/pnas.97.6.2832
28. Tsukada T, Yokoyama K, Arai T, Takemoto F, Hara S, Yamada A, Kawaguchi Y, Hosoya T, Igari J (1998) Evidence of association of the ecNOS gene polymorphism with plasma NO metabolite levels in humans. Biochem Biophys Res Commun 245(1):190–193. https://doi.org/10.1006/bbrc.1998.8267
29. Eechoute K, van der Veldt AA, Oosting S, Kappers MH, Wessels JA, Gelderblom H, Guchelaar HJ, Reyners AK, van Herpen CM, Haanen JB, Mathijssen RH, Boven E (2012) Polymorphisms in endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) predict sunitinib-induced hypertension. Clin Pharmacol Ther 92(4):503–510. https://doi.org/10.1038/clpt.2012.136
30. Garsen M, Rops AL, Li J, van Beneden K, van den Branden C, Berden JH, Rabelink TJ, van der Vlag J (2016) Endothelial nitric oxide synthase prevents heparanase induction and the development of proteinuria. PLoS One 11(8):e0160894. https://doi.org/10.1371/journal.pone.0160894
31. Niu W, Qi Y (2011) An updated meta-analysis of endothelial nitric oxide synthase gene: three well-characterized polymorphisms with hypertension. PLoS One 6(9):e24266. https://doi.org/10.1371/journal.pone.0024266
32. Garsen M, Lenoir O, Rops AL, Dijkman HB, Willemsen B, van Kuppevelt TH, Rabelink TJ, Berden JH, Tharaux PL, van der Vlag J (2016) Endothelin-1 induces proteinuria by heparanase-mediated disruption of the glomerular glycocalyx. J Am Soc Nephrol 27(12):3545–3551. https://doi.org/10.1681/ASN.2015091070
33. Momeni A, Chaleshtori MH, Saadatmand S, Kheiri S (2016) Correlation of endothelial nitric oxide synthase gene polymorphism (GG, TT and GT genotype) with proteinuria and retinopathy in Type 2 diabetic patients. J Clin Diagn Res 10(2):OC32–OC35. https://doi.org/10.7860/jcdr/2016/14975.7291
34. Casadei Gardini A, Marisi G, Faloppi L, Scarpi E, Foschi FG, Iavarone M, Lauletta G, Corbelli J, Valgiusti M, Facchetti F, Della Corte C, Neri LM, Tamberi S, Cascinu S, Scartozzi M, Amadori D, Nanni O, Tenti E, Ulivi P, Frassineti GL (2016) eNOS polymorphisms and clinical outcome in advanced HCC patients receiving sorafenib: final results of the ePHAS study. Oncotarget 7(19):27988–27999. https://doi.org/10.18632/oncotarget.8569
35. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C (2012) The role of nitric oxide on endothelial function. Curr Vasc Pharmacol 10(1):4–18
36. Kowalski TW, Fraga LR, Tovo-Rodrigues L, Sanseverino MT, Hutz MH, Schuler-Faccini L, Vianna FS (2016) New findings in eNOS gene and thalidomide embryopathy suggest pre-transcriptional effect variants as susceptibility factors. Sci Rep 6:23404. https://doi.org/10.1038/srep23404
37. Yoshimura M, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Nakamura S, Ito T, Mizuno Y, Harada E, Yasue H, Miyamoto Y, Saito Y, Nakao K (2000) A T-786–>C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene and coronary arterial vasomotility. Am J Cardiol 85(6):710–714
38. Sugimoto H, Shikata K, Matsuda M, Kushiro M, Hayashi Y, Hiragushi K, Wada J, Makino H (1998) Increased expression of endothelial cell nitric oxide synthase (ecNOS) in afferent and glomerular endothelial cells is involved in glomerular hyperfiltration of diabetic nephropathy. Diabetologia 41(12):1426–1434. https://doi.org/10.1007/s001250051088
39. Veelken R, Hilgers KF, Hartner A, Haas A, Bohmer KP, Sterzel RB (2000) Nitric oxide synthase isoforms and glomerular hyperfiltration in early diabetic nephropathy. J Am Soc Nephrol 11(1):71–79
40. Shoukry A, Shalaby SM, Abdelazim S, Abdelazim M, Ramadan A, Ismail MI, Fouad M (2012) Endothelial nitric oxide synthase gene polymorphisms and the risk of diabetic nephropathy in type 2 diabetes mellitus. Genet Test Mol Biomark 16(6):574–579. https://doi.org/10.1089/gtmb.2011.0218
41. Ezzidi I, Mtiraoui N, Mohamed MB, Mahjoub T, Kacem M, Almawi WY (2008) Association of endothelial nitric oxide synthase Glu298Asp, 4b/a, and −786T>C gene variants with diabetic

nephropathy. J Diabetes Complicat 22(5):331–338. https://doi.org/10.1016/j.jdiacomp.2007.11.011

42. Sun L, Mao D, Cai Y, Tan W, Hao Y, Li L, Liu W (2016) Association between higher expression of interleukin-8 (IL-8) and haplotype −353A/−251A/+678T of IL-8 gene with preeclampsia: a case–control study. Medicine (Baltimore) 95(52):e5537. https://doi.org/10.1097/MD.0000000000005537