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ORIGINAL PAPER

# Prognostic effect of VEGF gene variants in metastatic non-small-cell lung cancer patients

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## Abstract

**Introduction** Clinical and pathological characteristics are still considered prognostic markers in metastatic non-small-cell lung cancer (NSCLC) patients but they cannot explain all interindividual variability. Tumoral angiogenesis mediated by the vascular endothelial growth factor (VEGF) is critical for the progression and metastasis of the disease. We aimed to investigate the prognostic role of genetic variants within the VEGF pathway in patients with metastatic NSCLC.

**Materials and methods** We prospectively included 170 patients with metastatic NSCLC treated with first-line platinum-based chemotherapy. A comprehensive panel of single-nucleotide polymorphisms (SNPs) in genes belonging to the VEGF pathway (*VEGFA, VEGFR1/FLT1, VEGFR2/KDR, GRB2, ITGAV, KISS1, KRAS, PRKCE, HIF1α, MAP2K4, MAP2K6*, and *MAPK11*) were genotyped in blood DNA samples. SNPs were evaluated for association with overall survival (OS) and progression-free survival (PFS).

**Results** In multivariate analyses adjusted for patient characteristics, we found that *VEGFA* rs2010963 and *VEGFR2* rs2071559 were significantly associated with OS [Hazard Ratio (HR) 0.7 (0.5–0.9); $p = 0.026$ and HR 1.5 (1.1–2.3); $p = 0.025$, respectively]. Additionally, *ITGAV* rs35251833 and *MAPK11* rs2076139 were significantly associated with PFS [HR 2.5 (1.4–4.3; $p = 0.002$ and HR 0.6 (0.5–0.9); $p = 0.013$, respectively].

**Conclusion** Our findings reinforce the potential clinical value of germline variants in *VEGFA* and *VEGFR2* and show for the first time variants in *ITGAV* and *MAPK11* as promising prognostic markers in metastatic NSCLC patients receiving platinum-based chemotherapy.

**Keywords** VEGF · Genetic variants · Metastatic NSCLC · Prognostic factors

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## Introduction

Non-small-cell lung cancer (NSCLC) represents about 85% of lung cancer diagnoses and remains a major cause of cancer-related deaths worldwide [1]. Unfortunately, as most patients are diagnosed at an advanced stage, radical treatments are unfeasible and outcomes are poor [2]. The treatment strategy should take several factors into account, such as histology, molecular alterations, performance status (PS), age, comorbidities, and patient preferences. Chemotherapy with platinum doublets continues to be the standard of care in NSCLC patients without an actionable oncogenic driver, with clinical contraindications to immunotherapy and with PS of 0–2 [3]. Although prognosis depends mainly on clinicopathological characteristics, these factors alone are insufficient to predict the course of the disease [4]. Identifying novel prognostic biomarkers is thus needed to ameliorate patient outcomes.

Progression and metastasis of NSCLC is significantly promoted by angiogenesis triggered by the vascular endothelial growth factor (VEGF) signaling pathway [5, 6]. A major inducer of this process is hypoxia, mostly mediated by the hypoxia-inducible factor HIFα, which contributes to the synthesis of several proteins, including cellular receptors and proangiogenic factors. Among these, VEGF is the pivotal activator of this pathway through its binding to the membrane receptors VEGFR1 (FLT1) and VEGFR2 (KDR). VEGF overexpression has already been correlated with worse prognoses in lung cancer patients [4]. Some studies have reported that variants within VEGF-related genes regulate their transcription [7, 8]. Likewise, single-nucleotide polymorphisms (SNPs) in these genes affect tumor microvessel density and thus influence patient outcomes [9, 10].

Given the paramount importance of angiogenesis in NSCLC, we hypothesized that variants in genes of the VEGF signaling pathway could contribute to prognosis. Several studies have described associations between polymorphisms in VEGF-related genes and survival in advanced-stage NSCLC, although results reported to date are inconsistent [11–15]. Most of these studies have focused only on functional SNPs in the *VEGFA* gene, without including other key genes such as *VEGFR1* or *VEGFR2*. We aimed to evaluate the correlation of germline polymorphisms in the most relevant VEGF-related genes, with outcomes in a homogeneous cohort of metastatic NSCLC patients treated with first-line platinum-based chemotherapy.

## Materials and methods

### Study population

Between 2010 and 2014, one hundred and seventy metastatic NSCLC patients treated with first-line platinum-based chemotherapy at either Hospital de la Santa Creu i Sant Pau ($N=149$), Consorci Sanitari de Terrassa ($N=16$) or Hospital de Sant Pau i Santa Tecla ($N=5$) were prospectively included in the study. None of them harbored *EGFR*-activating mutations or *ALK* translocations. The following data were collected from electronic medical records: age, gender, PS according to ECOG (Eastern Cooperative Oncology Group) scale, tobacco exposure, histological subtype, disease stage IVA or IVB according to TNM/AJCC 7th edition [16], platinum-doublet chemotherapy regimen, and response according to RECIST (Response Evaluation Criteria in Solid Tumors) v1.1 [17].

The study was approved by the Institutional Ethics Committees and all the participants gave written informed consent for blood collection and genetic analyses.

### Genetic studies

We selected 24 SNPs with functional evidence in 12 VEGF-related genes (*VEGFA, VEGFR1/FLT1, VEGFR2/KDR, GRB2, ITGAV, KISS1, KRAS, PRKCE, HIF1α, MAP2K4, MAP2K6*, and *MAPK11*). The rationale for the selection of the SNPs has been detailed in previous studies [18–21]. All the SNPs presented a minor allele frequency (MAF) over 0.05 and an $r^2$ threshold of 0.8 in European population according to the 1000 Genomes project [22]. Table 1 includes more detailed information about the selected polymorphisms.

Genomic DNA was automatically extracted from peripheral whole-blood samples (Autopure, Qiagen, Hilden, Germany). The SNPs were analyzed by real-time PCR using TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) and 48.48 dynamic arrays on the BioMark™ system (Fluidigm, San Francisco, CA, USA). More than 99.8% of cases were successfully genotyped.

### Statistical analyses

Hardy–Weinberg equilibrium was assessed for each analyzed SNP using a Chi-square test. Codominant, dominant, and recessive models of inheritance were considered to assess associations with outcome variables whenever appropriate.

The primary endpoint was overall survival (OS) and the secondary endpoint was progression-free survival (PFS). OS was defined as the time from diagnosis until death from any cause or last clinical follow-up. PFS was defined as the time from treatment initiation until disease progression or death, whichever occurred first. Considering hazard ratios (HR) of 1.7 for OS, and adopting two-sided $\alpha=0.05$ and $\beta=0.20$, we estimated 138

**Table 1** Selected putative functional polymorphisms in VEGF-related genes

| Gene symbol | refSeq | Label | Relevant SNPs in LD | MAF | Minor allele |
|---|---|---|---|---|---|
| *GRB2* | rs7219 | c.*1081G > A | rs8079197 | 0.26 | G |
| *HIF1α* | rs11549465 | c.1816C > T | | 0.10 | T |
| *ITGAV* | rs35251833 | c.2791-163G > A | rs1839123, rs9333289 | 0.31 | A |
| *KISS1* | rs71745629 | c.417delA | | 0.22 | – |
| *KRAS* | rs61764370 | c.*2505T > G | | 0.10 | G |
| | rs10842513 | c.112-4312A > G | rs10505980 | 0.09 | A |
| | rs12813551 | c.111 + 3429G > A | | 0.40 | G |
| | rs1137282 | c.519T > C | | 0.22 | C |
| *MAP2K4* | rs3826392 | c.-1300G > T | rs3809728 | 0.25 | G |
| *MAP2K6* | rs11656130 | g.67400446T > G | | 0.45 | G |
| | rs2716191 | g.67536322T > C | | 0.48 | C |
| *MAPK11* | rs2076139 | c.756A > G | | 0.26 | A |
| *PRKCE* | rs4953299 | c.1264-274T > C | | 0.24 | C |
| *VEGFA* | rs699947 | c.-2055A > C | rs833061, rs833070 | 0.50 | A |
| | rs1570360 | c.-614A > G | | 0.32 | A |
| | rs2010963 | c.-94C > G | rs3024997 | 0.31 | C |
| | rs3025039 | c.*237C > T | rs3025040 | 0.12 | T |
| *VEGFR1* | rs9582036 | c.3635 + 319G > T | rs9554316, rs7993418 | 0.27 | G |
| | rs7996030 | c.3636-273T > C | | 0.20 | T |
| | rs9513070 | c.3815 + 976C > T | | 0.41 | C |
| *VEGFR2* | rs2305948 | c.889G > A | | 0.09 | A |
| | rs1551641 | g.55993915C > T | rs10013228 | 0.30 | T |
| | rs2071559 | c.-906T > C | rs7667298 | 0.49 | C |
| | rs1870377 | c.1416A > T | | 0.23 | A |

*GRB2* growth factor receptor-bound protein 2, *HIF1α* hypoxia-inducible factor 1, alpha subunit, *ITGAV* integrin, alpha V; *KISS1* KiSS-1 metastasis-suppressor, *KRAS* Kirsten rat sarcoma viral oncogene homolog, *MAP2K4* mitogen-activated protein kinase kinase 4, *MAP2K6* mitogen-activated protein kinase kinase 6, *MAPK11* mitogen-activated protein kinase 11, *PRKCE* protein kinase C, epsilon; *VEGFA* vascular endothelial growth factor A, *VEGFR1* or *FLT1* fms-related tyrosine kinase 1, *VEGFR2* or *KDR* kinase insert domain receptor, *MAF* minor allele frequency (1000 Genomes Project, European population; accession date: 21/08/18), *LD* Linkage disequilibrium; refSeq, reference sequence

Label according to the accession numbers: NM_002086.4 (*GRB2*), NM_001243084.1 (*HIF1α*), NM_001144999.2 (*ITGAV*), NM_002256.3 (*KISS1*), NM_004985.4 (*KRAS*), NM_001281435.1 (*MAP2K4*), NC_000017.10 (*MAP2K6*), NM_002751.6 (*MAPK11*), NM_005400.2 (*PRKCE*), NM_001025366.2 (*VEGFA*), NM_002019.4 (*VEGFR1*), NC_000004.11 (rs1551641, *VEGFR2*), and NM_002253.2 (*VEGFR2* SNPs except rs1551641)

events would be needed to detect the prognostic effect of the genetic variants (Schoenfeld). To achieve this objective, the study was continued until April 2017. For OS and PFS analyses, survival curves and survival medians were estimated at the 95% confidence level using the Kaplan–Meier method, and differences in survival were analyzed with the log-rank test. A Cox proportional hazards regression model was adjusted for multivariate analysis of OS and PFS, including all significant polymorphisms in the univariate analysis and ECOG PS, tobacco exposure, histological subtype, and disease stage. The results were considered statistically significant when p-values were below 0.05. All statistical analyses were performed using SPSS (version 24.0, IBM).

## Results

### Clinical results

Table 2 summarizes the patients' baseline clinical and pathological characteristics. After a median follow-up of 14.7 (range 1.0–75.0) months, 160 (94.1%) patients had progressed and 140 (82.4%) had died. The median OS was 14.8 [95% confidence interval (CI) 12.0–17.6] months, and the median PFS was 6.0 [95% CI 4.7–7.2] months.

Overall survival differed significantly according to ECOG PS and tobacco exposure. Patients with an ECOG PS of 0 had a median OS of 22.8 months [95% CI 0.0–60.2] compared to 12.7 months [95% CI 9.0–16.3] in patients with an ECOG PS of 1, and to 12.8 months [95%

**Table 2** Patient and tumor characteristics ($N = 170$)

| Characteristics | *N* | % |
|---|---|---|
| Age (years) | | |
| Median | 63 | |
| Range | [32,86] | |
| Gender | | |
| Male | 131 | 77.1 |
| Female | 39 | 22.9 |
| Performance status (ECOG) | | |
| 0–1 | 132 | 77.6 |
| 2 | 38 | 22.4 |
| Tobacco exposure | | |
| No | 24 | 14.1 |
| Yes | 146 | 85.9 |
| Histological subtype | | |
| Adenocarcinoma | 118 | 69.4 |
| Squamous-cell carcinoma | 37 | 21.8 |
| Large-cell carcinoma | 15 | 8.8 |
| Disease stage | | |
| IVA | 37 | 21.8 |
| IVB | 133 | 78.2 |
| Treatment | | |
| Platinum plus pemetrexed | 59 | 34.7 |
| Platinum plus gemcitabine | 56 | 32.9 |
| Platinum plus vinorelbine | 28 | 16.5 |
| Platinum plus taxanes | 27 | 15.9 |
| Response | | |
| Complete response | 5 | 2.9 |
| Partial response | 69 | 40.6 |
| Stable disease | 59 | 34.7 |
| Disease progression | 37 | 21.8 |

*ECOG* Eastern Cooperative Oncology Group

CI 8.7–16.9] in patients with an ECOG PS of 2 ($p = 0.01$). Regarding tobacco exposure, patients who smoked had a median OS of 12.7 months [95% CI 10.4–14.9), whereas non-smokers had a median OS of 22.8 months [95% CI 15.7–29.9] ($p = 0.027$). In the multivariate analysis including all the clinical variables, both ECOG PS and tobacco exposure retained their statistical significance ($p = 0.01$ and $p = 0.04$, respectively).

We also found a significant difference between PFS and ECOG PS. Patients with an ECOG PS of 0 had a median PFS of 12.2 months [95% CI 7.8–16.6] compared to 5.9 months [95% CI 5.3–6.5] in patients with an ECOG PS of 1, and to 5.1 months [95% CI 0.5–6.7] in patients with an ECOG PS of 2 ($p = 0.008$). In the multivariate analysis, this association remained statistically significant ($p = 0.004$).

## Genetic variants and survival

All the genotypic frequencies were in Hardy–Weinberg equilibrium except for rs11656130 T>G (*MAP2K6*) and rs61764370 T>G (*KRAS*) variants, which were therefore excluded from the analysis.

In the univariate analyses, four SNPs showed a statistically significant association with OS: rs2010963 G>C (*VEGFA*), rs2071559 C>T (*VEGFR2*), rs10842513 G>A (*KRAS*), and rs3826392 T>G (*MAP2K4*) (Table 3). For the rs2010963 variant, patients with the GC genotype had a longer median OS (18.9 months) than patients with the GG (12.5 months) and CC genotypes (12.3 months) ($p = 0.009$) (Fig. 1a). For the rs2071559 variant, patients with the CC genotype achieved a longer median OS than patients carrying the T allele for the variant (17.2 vs. 12.8 months) ($p = 0.024$ in a dominant model) (Fig. 1b). For the rs10842513 variant, patients with the GG genotype had a longer median OS than A allele carriers (15.6 vs. 8.8 months) ($p = 0.024$ in a dominant model). For the rs3826392 variant, patients with the TT genotype had a longer median OS (16.5 months) than patients with the TG and GG genotypes (12.7 and 9.9 months, respectively) ($p = 0.042$). Non-significant associations between genetic variants and OS are shown in the Supplementary Table. In the multivariate analysis, two of these SNPs showed statistical significance considering a dominant model of inheritance: *VEGFA* rs2010963 (HR 0.7; 95% CI 0.5–0.9; $p = 0.026$) and *VEGFR2* rs2071559 (HR 1.5; 95% CI 1.1–2.3; $p = 0.025$).

In the univariate analyses, three variants were significantly associated with PFS: rs9513070 T>C (*VEGFR1*), rs35251833 G>A (*ITGAV*), and rs2076139 G>A (*MAPK11*) (Table 3). For the rs9513070 variant, patients carrying the C allele had a longer median PFS than those with the TT genotype (7.1 vs. 5.0 months) ($p = 0.025$ in a dominant model). For the rs35251833 variant, patients carrying the G allele presented a longer median PFS than patients with the AA genotype (6.3 vs. 4.0 months) ($p = 0.027$ in a recessive model). For the rs2076139 variant, patients carrying the A allele had a longer median PFS than patients with the GG genotype (7.6 vs. 5.2 months) ($p = 0.006$ in a dominant model). The supplementary table summarizes the non-significant associations between genetic variants and PFS. In the multivariate analysis, two of the variants retained their statistical significance: *ITGAV* rs35251833 (HR 2.5; 95% CI 1.4–4.3; $p = 0.002$) and *MAPK11* rs2076139 (HR 0.6; 95% CI 0.5–0.9; $p = 0.013$), considering a recessive and a dominant model, respectively.

**Table 3** Univariable analyses of VEGF genetic variants and their significant association with overall survival or progression-free survival

| Gene | SNP | *N* | OS | | | PFS | | |
|---|---|---|---|---|---|---|---|---|
| | | | Median OS, months (95% CI) | Hazard ratio (95% CI) | *P* value | Median PFS, months (95% CI) | Hazard ratio (95% CI) | *P* value |
| *VEGFA* | rs2010963 | | | | | | | |
| | GG | 77 | 12.5 (8.20–16.72) | 1 (reference) | **0.009** | 5.7 (4.49–7.00) | 1 (reference) | 0.181 |
| | GC | 70 | 18.9 (13.68–24.09) | 0.78 (0.48–1.27) | | 6.8 (5.16–8.42) | 0.78 (0.48–1.26) | |
| | CC | 23 | 12.3 (8.33–16.33) | 0.50 (0.30–0.83) | | 5.5 (4.24–6.78) | 0.64 (0.39–1.05) | |
| | GC + CC | 93 | 15.7 (11.29–20.19) | 1.32 (0.95–1.85) | 0.100 | 6.0 (4.32–7.62) | 1.10 (0.80–1.50) | 0.553 |
| *VEGFR1* | rs9513070 | | | | | | | |
| | TT | 57 | 12.7 (8.14–17.17) | 1 (reference) | 0.348 | 5.0 (3.20–6.71) | 1 (reference) | **0.022** |
| | TC | 83 | 17.0 (11.90–22.13) | 0.76 (0.19–3.15) | | 7.7 (6.70–8.71) | 1.43 (0.35–5.90) | |
| | CC | 28 | 13.8 (8.10–19.57) | 0.57 (0.14–2.33) | | 5.0 (3.74–6.29) | 0.87 (0.21–3.58) | |
| | TC + CC | 111 | 15.6 (12.03–19.25) | 1.25 (0.88–1.78) | 0.210 | 7.1 (5.81–8.49) | 1.45 (1.05–2.02) | **0.025** |
| *VEGFR2* | rs2071559 | | | | | | | |
| | CC | 52 | 17.2 (12.76–21.61) | 1 (reference) | 0.079 | 7.2 (5.67–8.76) | 1 (reference) | 0.415 |
| | CT | 80 | 12.9 (8.00–17.77) | 0.66 (0.41–1.06) | | 5.4 (4.49–6.26) | 0.93 (0.60–1.44) | |
| | TT | 38 | 12.4 (6.22–18.50) | 1.03 (0.68–1.56) | | 6.0 (3.59–8.41) | 1.17 (0.78–1.76) | |
| | CT + TT | 118 | 12.8 (8.95–16.63) | 0.65 (0.45–0.95) | **0.024** | 5.5 (4.65–6.37) | 0.83 (0.60–1.17) | 0.289 |
| *ITGAV* | rs35251833 | | | | | | | |
| | GG | 94 | 16.3 (12.72–19.94) | 1 (reference) | 0.598 | 6.0 (4.67–7.26) | 1 (reference) | **0.046** |
| | GA | 59 | 14.5 (10.37–18.62) | 0.75 (043–1.31) | | 7.1 (5.04–9.26) | 0.61 (0.36–1.04) | |
| | AA | 17 | 10.5 (3.30–17.75) | 0.79 (0.44–1.41) | | 4.0 (2.51–5.56) | 0.50 (0.28–0.87) | |
| | GG + GA | 153 | 15.6 (12.52–18.69) | 0.76 (0.45–1.31) | 0.326 | 6.3 (5.15–7.38) | 0.56 (0.33–0.95) | **0.027** |
| *KRAS* | rs10842513 | | | | | | | |
| | GG | 150 | 15.6 (12.75–18.53) | 1 (reference) | **0.024** | 6.0 (4.74–7.20) | 1 (reference) | 0.490 |
| | GA + AA | 20 | 8.8 (4.52–12.99) | 0.57 (0.34–0.93) | | 5.3 (1.65–8.91) | 0.84 (0.51–1.38) | |
| *MAP2K4* | rs3826392 | | | | | | | |
| | TT | 99 | 16.5 (14.31–18.61) | 1 (reference) | **0.042** | 5.9 (4.60–7.27) | 1 (reference) | 0.989 |
| | TG | 63 | 12.7 (7.98–17.33) | 0.40 (0.19–0.84) | | 6.0 (3.98–8.02) | 0.98 (0.45–2.12) | |
| | GG | 8 | 9.9 (8.90–10.90) | 0.41 (0.19–0.89) | | 7.7 (0.00–19.99) | 1.01 (0.46–2.20) | |
| *MAPK11* | rs2076139 | | | | | | | |
| | GG | 112 | 14.8 (11.59–18.05) | 1 (reference) | 0.574 | 5.2 (3.99–6.37) | 1 (reference) | **0.024** |
| | GA | 54 | 13.8 (9.63–18.05) | 1.80 (0.57–5.70) | | 7.5 (6.97–8.05) | 1.57 (0.58–4.28) | |
| | AA | 4 | 22.5 (0.10–44.95) | 1.67 (0.52–5.38) | | 8.8 (5.29–12.29) | 0.99 (0.35–2.75) | |
| | GA + AA | 58 | 14.5 (8.45–20.53) | 1.12 (0.79–1.60) | 0.520 | 7.6 (6.76–8.38) | 1.60 (1.14–2.24) | **0.006** |

The values highlighted in bold are statistically significant ($P < 0.05$)

*OS* overall survival, *PFS* progression-free survival, *SNP* single-nucleotide polymorphism

## Discussion

Variants in genes belonging to the VEGF pathway were found to be associated with outcomes in metastatic NSCLC patients treated with first-line platinum-based chemotherapy. The multivariate analyses after adjusting for the most relevant clinical and pathological parameters showed that polymorphisms in the VEGF pathway were significantly associated with OS, rs2010963 (*VEGFA*) and rs2071559 (*VEGFR2*), and with PFS, rs35251833 (*ITGAV*) and rs2076139 (*MAPK11*).

A large body of research suggests that polymorphisms within the main VEGF-related genes can predict NSCLC outcomes, although their clinical utility has not yet been demonstrated [11–15, 19, 20, 23–27]. Some of the most interesting findings to date concern the *VEGFA* rs2010963 (c.−634G>C, +405G>C). In 2008, Heist et al. conducted a study in a cohort of 462 stage IA-IIB NSCLC patients and observed that C allele carriers of the rs2010963 presented longer OS ($p = 0.006$) [25]. Later, through univariate analysis, de Mello et al. found that this SNP was also significantly associated with OS in stage I–IV NSCLC patients

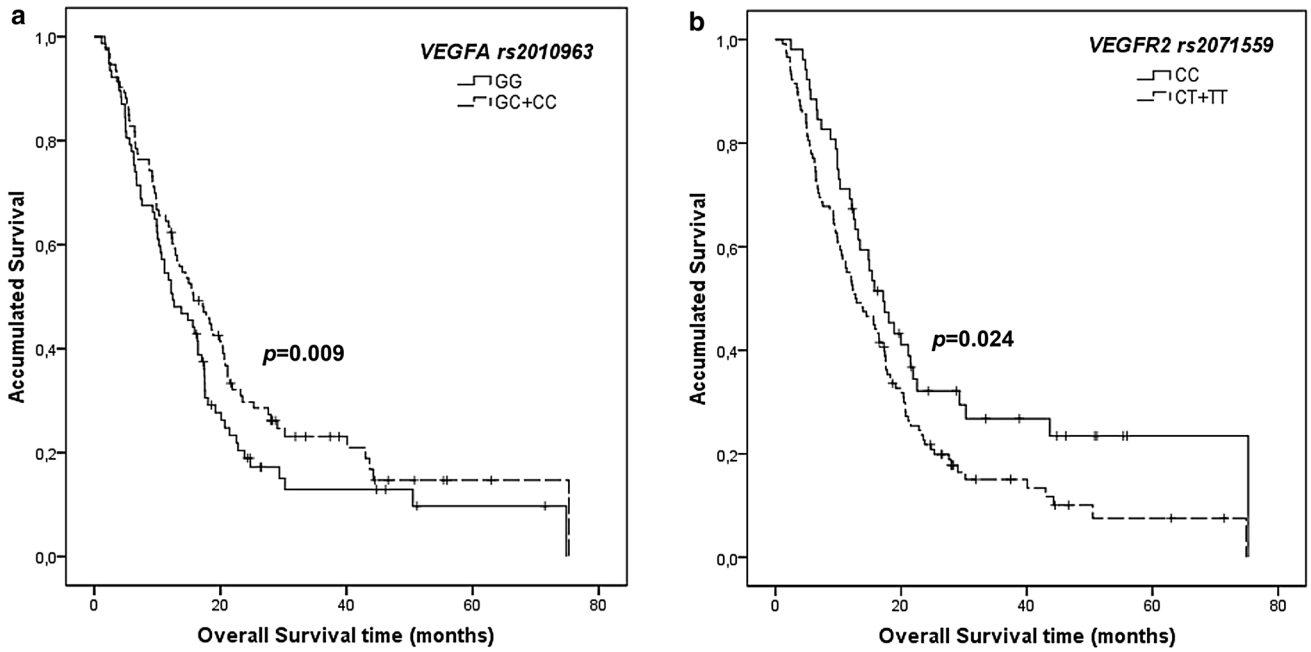


**Fig. 1** OS by genotype for patients with the *VEGFA* rs2010693 polymorphism (**a**) and *VEGFR2* rs2071559 polymorphism (**b**). OS overall survival, *VEGFA* vascular endothelial growth factor A, *VEGFR2* or *KDR* kinase insert domain receptor

($p = 0.042$) [12]. Conversely, other studies performed in NSCLC patients did not find any correlation between this SNP and OS [11, 13–15]. Other findings of note include the 2012 study by Eng et al. In their systematic review and meta-analysis [26], updated in 2013 [27], these authors evaluated the role of polymorphisms in the VEGF pathway as prognostic and predictive factors in cancer, including NSCLC. They reported that harboring the C allele of rs2010963 conferred a significant protective effect on OS regardless of the tumor type ($p = 0.007$). Additionally, this allele correlated with better overall response to bevacizumab in combination with platinum-based chemotherapy ($p = 0.038$) in a cohort of 303 patients with advanced or recurrent non-squamous NSCLC from the ABIGAIL study [28]. The functional effect of this variant on VEGF expression remains inconsistent. Whereas two studies reported that the GG genotype implied higher VEGF production [8, 29], another study carried out in NSCLC described opposite results [7]. In our study, we observed that C allele carriers of rs2010963 presented longer OS, a finding in keeping with the results reported by previous studies [25, 26]. These findings strengthen the hypothesis that rs2010963 could predict NSCLC outcomes, although more research is required.

As for the association between *VEGFR2* rs2071559 (c.−906T>C, −604T>C) and survival in NSCLC patients, few studies have been conducted to date. Uzunoglu et al. found that NSCLC adenocarcinoma patients who underwent surgical resection with the TT genotype presented better OS than patients with the CC genotype ($p = 0.024$) [24]. In a subsequent study conducted in a cohort of 350 inoperable stage I–IV primary NSCLC patients receiving radiotherapy, the C allele was significantly associated with poorer OS ($p = 0.002$) and PFS ($p = 0.009$) [23]. However, in the ABIGAIL study, no associations were found between the variant and OS or PFS [28]. In silico and in vitro functional analyses showed that rs2071559 correlated with VEGFR2 mRNA expression in NSCLC tumors, which could be explained by the fact that the variant introduces a putative IKZF2 (IKAROS Family Zinc Finger 2) transcription binding site in the promoter region [9]. In the present study, we found that metastatic NSCLC patients with the CC genotype for the polymorphism had a greater OS. The inconsistency of the results so far published could be due to the heterogeneity in patient populations tested, as it has been reported that VEGFR2 protein expression differs between early- and advanced-stage NSCLC [9]. Consequently, in advanced-stage NSCLC, further research is needed to confirm the relevance of the rs2071559 variant as a prognostic marker.

*ITGAV* rs35251833 was also found to be clinically relevant in the present study. ITGAV is a cell surface adhesion receptor involved in cell survival, proliferation, and invasion, as well as in tumor growth and angiogenesis, by interacting with the extracellular matrix. Thus, genetic variants in *ITGAV* are likely to modulate NSCLC prognosis. Yi et al. performed a study in a cohort of 301 Chinese lung cancer patients treated with thoracic radiation in which they investigated the association of functional polymorphisms in *ITGAV* and *ITGB6* integrin genes with the risk of radiation pneumonitis [30]. In their set

of polymorphisms, they included the rs1839123, which is in high LD with rs35251833 ($D'$: 1.0, $r^2$: 0.99 in European and Asian populations; data from Phase 3 of the 1000 Genomes Project), and found no association with the variant. More recently, we analyzed the correlation of the variant with survival in a cohort of stage I–III NSCLC patients treated with complete surgical resection, and obtained negative results [19]. The variant is an intronic SNP associated with transcriptional regulation, which suggests functional relevance. In the present study, we found that the G allele of rs35251833 correlated with longer PFS in metastatic NSCLC patients treated with first-line platinum-based chemotherapy. Apart from the results herein reported, there are no data showing significant associations of rs35251833 with outcomes in NSCLC, although it should be noted that little research has been conducted in this area. Moreover, two previous studies conducted in colon cancer patients showed better outcomes in patients harboring the G allele [18, 21]. Based on the findings of our group, we propose the variant as a potential outcome biomarker, worthy of validation.

Finally, almost no data are available regarding the potential value of the *MAPK11* rs2076139 variant as a biomarker of survival in NSCLC. MAPK11 participates in the MAPK signaling pathway, which modulates angiogenesis and cancer outcomes. In lung cancer, the previously mentioned study by Sullivan et al. conducted in early-stage NSCLC patients showed no association of the rs2076139 variant with prognosis [19]. The analyzed SNP is located in exon 9 and causes a synonymous substitution (p.Ser252=) of unknown pathogenicity, although it has been associated with mRNA levels [31]. In the present study, we found that metastatic NSCLC patients carrying the A allele presented better PFS. Our data suggest a possible role of the *MAPK11* rs2076139 variant in predicting survival in metastatic NSCLC patients, although additional studies are needed to validate this result.

The present study adds support to the utility of *VEGF*-related polymorphisms as prognostic biomarkers in metastatic NSCLC patients. We conducted a comprehensive evaluation of putative functional polymorphisms in the VEGF pathway genes, but more in vitro functional analyses are needed to provide further insight into their pathogenicity. It should be noted that our findings could be limited by the relatively small number of patients included. However, as far as we know, this is the largest reported prospective cohort of metastatic NSCLC patients treated with a platinum-based chemotherapy regimen in which associations between SNPs in the VEGF pathway and survival have been analyzed.

## Conclusions

This prospective study provides additional evidence concerning the association of functional germline polymorphisms in *VEGFA* and *VEGFR2* genes with prognosis. Moreover, it shows for the first time that variants in *ITGAV* and *MAPK11* genes involved in the angiogenesis process predict clinical outcome in metastatic NSCLC patients.

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## Compliance with ethical standards

**Conflict of interest** The authors declared no conflict of interest.

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