The Role of Vascular Endothelial Growth Factor (VEGF) SNPs as Predictive and Prognostic Markers for Major Solid Tumors

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Abstract

Angiogenesis is crucial for development and metastasis of tumors and VEGF is a key mediator of this process. The importance of VEGF in tumorigenesis and tumor progression makes it an attractive target for the development of anticancer therapies. Inhibition of angiogenesis has shown promising clinical efficacy; however, not all patients treated with anti-angiogenic agents get benefited. Some patients are predisposed to refractory disease while others develop resistance after initial response. Patients may also have different severity of drug-related adverse events. Optimization of drug administration based on disease status and individual responsiveness is important in limiting the treatment failure and minimization of side-effects. Single nucleotide polymorphisms (SNPs) in *VEGF* may alter VEGF protein concentrations, influence the process of angiogenesis and may relate to inter-individual variation in the risk and progression of selected tumors, and their resistance to treatments. This review examines the role of SNPs in *VEGF* gene as predictive and prognostic markers for major solid tumors, including the breast, non-small cell lung (NSCLC), colorectal (CRC) and prostate cancers. Selected *VEGF* SNPs appear to be associated with risk of these cancers; however, there is lack of unanimity in findings, in part influenced by differences in study design and analysis.

Keywords

VEGF; gene; SNP; cancer; breast; lung; colon; prostate

Introduction

Antiangiogenic therapies have improved the clinical outcome of selected tumors by inhibition of tumor growth via restriction of new blood vessel formation. As tumor grows, the lack of sufficient blood supply creates an hypoxic environment, stimulating the release of factors like hypoxia inducible factor (HIF)−1α, which induces angiogenesis by activating the transcription

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of VEGF (1). VEGF and its receptors are frequently overexpressed in human tumors, including the breast, non-small cell lung, colorectal and prostate cancers (2–5). VEGF and associated signaling pathways have been the target for many newly developed anticancer drugs. These agents have shown promising efficacy; however, they are often very expensive and some patients experience drug resistance, limited activity, and severe toxicities. There is a strong need for identification of markers enabling *a priori* selection of patients who are likely to benefit from these agents. These markers might also carry prognostic value and may help in decisions about the overall treatment approach to manage more or less aggressive tumors with varying degrees of angiogenic involvement. This review evaluates the role of *VEGF* SNPs in predicting the cancer susceptibility, progression, and anti-VEGF therapeutic response in subjects with major solid tumors including the breast, non-small cell lung, colorectal and prostate cancers. For these tumors anti-VEGF or anti-VEGFR treatments are either approved or in advance stages of clinical development. We also performed meta-analysis, where methods of DerSimonian and Laird (6) were used to test the homogeneity of odds ratio across studies and for calculation of pooled OR and their confidence intervals. Standard error estimates for individual OR were calculated using exact methods from published genotype frequencies or were derived from published confidence intervals.

VEGF gene

The *VEGF* gene is located on chromosome 6 at location 6p21.1 (7). Its coding region spans approximately 14 kilobases and consists of 8 exons (8,9). Numerous SNPs in the promoter, 5'-, and 3'- untranslated regions (UTR), are present in *VEGF*. Some of the more frequent SNPs are shown in Figure 1 and their frequencies in control population are summarized in Table 1. The 5'- and 3'- UTR contain key regulatory elements which are sensitive to hypoxia (10), and contributes to high variability in VEGF production among tissues (11). For example −634G>C SNP in the 5'-UTR of *VEGF* affects the protein translation efficiency (12), and 936C>T SNP in the 3'-UTR influences the circulating plasma concentrations (13) and tumor tissue expression of VEGF (14). However, it is likely that only a small number of these polymorphisms and haplotypes (linearly linked SNPs) actually have a functional effect on VEGF translation, whereas others act as proxies (15).

VEGF SNPs in breast cancer

VEGF has shown to be important for development, invasiveness and metastasis of breast cancer in both preclinical and clinical settings (16). Higher levels of VEGF have shown to be related with adverse prognosis, decreased overall survival and resistance to hormonal therapy (17–19). High microvessel density (MVD), a marker of VEGF expression and local activity, increases the likelihood of metastatic disease (20) and acts as a prognostic indicator for relapse-free and overall survival in node-negative breast cancer patients (21). These data suggest that SNPs in *VEGF* may play a substantial role in development and progression of breast cancer; studies evaluating this hypothesis are summarized in Table 2.

A case-control study, with 500 subjects in each group, evaluating the role of the germline *VEGF* 936C>T SNP in Austrian subjects, found that subjects with variant 936T allele were at reduced risk of breast cancer (22). However, the 936C>T SNP was not related to prognostic factors such as tumor size, histological grades, lymph node status, estrogen and progesterone receptor status or age at diagnosis (22). In a small subset of 21 healthy, non-smoking postmenopausal women, the 936T allele was associated with significantly lower plasma concentrations of VEGF, which may partially explain, the reduced risk of cancer associated with this allele (22). A smaller study from the UK, including 144 cases and 263 controls, evaluated the effect of SNPs in the promoter regions of various cytokines, including the *VEGF* −1154G>A transition, on risk and prognosis of breast cancer. The −1154G>A allele

was not associated with risk of breast cancer; however, carriers of heterozygous −1154AG genotype displayed a trend towards favorable prognosis (23). A larger case-control study including 571 patients with familial breast cancer from Germany and Poland, and 974 patients with sporadic breast cancer from Sweden failed to find a relation between *VEGF* SNPs (−2578C>A, −1154G>A, −634G>C, and 936C>T) or their haplotypes and risk of breast cancer (24). This finding is in contrast with the earlier report (22) suggesting protective role of 936T allele. However, patients with the −634CC genotype and −2578C/−634 C haplotype had larger, more histologically advanced tumors while −2578AA and −2578A/−634G had low histological grade tumors, suggesting that these SNPs might act as genetic marker for tumor aggressiveness (24).

Two different publications (25,26) evaluated data from the Shanghai Breast Cancer Study, a large population based case-control study to evaluate the impact of germline *VEGF* −1498C>T, −634G>C, and 936C>T SNPs on survival and risk of breast cancer in Chinese women. Based on data from 1133 cases and 1233 age-matched controls, the −1498 CC allele was associated with shorter overall and disease-free survival (age adjusted hazard ratio of 1.5 for both) (25), but had no association with risk of breast cancer (26). The carriers of the −634GG allele had significantly shorter overall survival than non-carriers with an age adjusted hazard ratio of 1.6 but the disease-free survival and risk were not different between these groups (25,26), while previously the complementary allele −634CC was shown to be related with advanced tumors (24). The 936C>T SNP was not related to overall or disease-free survival (25), but the 936TT genotype carriers were at reduced risk of cancer (OR, 0.65) (26), agreeing with Krippl et al. (22) despite of differences in ethnicity. The subset analysis based on menopausal status explained that the protective role of the 936TT genotype was restricted to pre-menopausal women (OR, 0.45). Haplotype analysis demonstrated that −1498T/−634C/936C was related with longer overall survival (hazard ratio (HR), 0.57), and pre-menopausal women with −1498T/−634G/936T were at reduced risk of breast cancer (OR, 0.47) compared to individuals with the reference −1498C/−634G/936T haplotype (25,26).

A study evaluating the impact of four previously studied (24) *VEGF* SNPs (−2578C>A, −1154G>A, −634G>C and 936C>T) in Caucasian population from a different geographic location (i.e. USA), including 501 cases and 504 controls, also failed to find any association between these SNPs and the risk of breast cancer (27), disregarding the spread of disease. However, subgroup analysis in patients with invasive (n=380) and *in-situ* (n=107) breast cancer showed higher risk of invasive, but not of *in-situ*, cancer in carriers of −2578CC (OR, 1.46) and −1154GG (1.64) genotypes (27). Conversely, the 936C allele was not associated with invasive cancer, but was related to reduced risk of *in-situ* breast cancer (OR, 0.59) (27). Notably, this finding is in contrast with the majority of previous studies where patients with the 936T allele had lower risk of breast cancer (22,26). The −634G>C SNP had no association with risk of either *in-situ* or invasive breast cancer. These results demonstrate the possible differences in genetic markers based on stage of disease (i.e., localized or metastatic). Haplotype analysis showed that one or two copies of −2578A/−1154A/−634G resulted in lower risk of invasive breast cancer compared to zero-copy carriers (OR, 0.69 and 0.62, respectively). Assessment of promoter diplotypes suggested no significant association with risk of invasive breast cancer (27). Another case-control study in 500 Caucasian patients and 500 matched controls analyzed the effect of germline *VEGF* −1498C>T, −634G>C, −7C>T and 936C>T SNPs on risk and severity of breast cancer (assessed by tumor size, tumor grade, nodal involvement, vascular invasion and oestrogen receptor status), survival of patients, circulating levels of VEGF and VEGF expression in tumor (28). Singularly, none of these SNPs were related to risk, but patients carrying the −1498T/−634C/−7C/936C haplotype were at lower risk of breast cancer than non-carriers. Except for −7C>T transition, which predicted longer overall survival, no other SNPs were related with survival (28). The serum and plasma VEGF levels and tumor VEGF expression were not related with any of these four SNPs (28). A large

case-control study including 804 breast cancer patients and 804 age-matched healthy volunteers from Austria found that germline *VEGF* −2578C>A, −2489C>T, −1498C>T, −634G>C, −7C>T, 936C>T and 1612G>A SNPs or their haplotypes were not related with risk of breast cancer (29). However the −634C allele was related with small tumor size (29), contrasting an earlier report (24) showing −634CC allele's relation with high tumor aggressiveness. A small case-control study with 60 patients and 60 age matched healthy volunteers found no carriers of the 936TT genotype in two groups, and the frequency of the 936CT genotype was significantly higher in patients compared to controls (30). The 936C>T SNP had no association with clinicopathological characteristics of the patients(30).

An epidemiological study with predominantly Caucasian subjects, including 715 controls and 520 breast cancer cases, evaluated the impact of alleles in seven genes, including the *VEGF* −2578C>A, −1498C>T, −634G>C, −1154G>A, and 936C>T SNPs on risk of breast cancer. The −2578AA and −1498CC genotypes had higher risk of breast cancer, independent of non-genetic risk factors estimated by the Gail model (respective adjusted OR, 1.99 and 2.01), while other *VEGF* SNPs had no association with disease risk (31). Finally, a case-control study in Polish Caucasian subjects, including 319 breast cancers, 146 ovarian cancers, and 290 unaffected controls, investigated the effect of the 936C>T SNP in modifying the hereditary risk of breast and ovarian cancer in women harboring a mutated BRCA1 gene (32). Carriers of the *VEGF* 936 T allele and BRCA1 mutation had reduced risk of breast cancer (OR, 0.63 for CT+TT genotype) but the risk of ovarian cancer was not affected (32). These findings suggest a protective role for the 936T allele in women at risk of breast cancer carrying BRCA1 mutations.

Recently two studies (33,34) evaluated the relationship of VEGF plasma levels or somatic *VEGF* and *VEGFR*-2 SNPs with treatment efficacy or toxicity. One of them compared the effect of paclitaxel with paclitaxel and bevacizumab combination in metastatic breast cancer by enrolling 183 patients in each arm (33). The *VEGF* −2578AA had longer median overall survival (HR 0.58) compared to the −2578CA+CC genotype in the combination arm (33). Similarly −1154A allele had longer overall survival (HR 0.62) in the combination arm, which increased additively with increase in number of A alleles (33). *VEGF* −634CC and −1498TT had significantly less likelihood of developing grade 3 or 4 hypertension in the combination arm compared to −634 GC+GG and −1498 CT+CC respectively (33). The *VEGF* 936C>T, *VEGFR-2* 889G>A and *VEGFR-2* 1416A>T had no significant relationship with either the efficacy or toxicity. Another study enrolled 56 breast cancer patients to examine the role of plasma VEGF levels as predictor of treatment outcome with bevacizumab and vinorelbine therapy (34). Lower levels of baseline VEGF predicted longer progression free survival (34).

In conclusion, *VEGF* −634G>C (24,26–29,31), −7C>T (26,28,29), and −2489C>A (26,29) SNPs were not related with risk of breast cancer. *VEGF* −1154G>A had no relation with overall risk of breast cancer (23,24,27,31), however the G allele was related with increased risk of invasive disease (23,27,31). *VEGF* −1498C>T was shown to have no relation (26,28,29) or predicted higher risk of breast cancer (31). Results for *VEGF* −2578C>A were inconsistent, i.e., no association with risk (23,24,27,29) and higher risk of invasive disease with the C allele (23,27,31) or A allele (31). For the 936C>T SNP, three studies have shown a protective role of the 936T allele(22,26,32), four failed to find any correlation with risk (24,28,29,31) and one found 936 C as a protective allele for *in-situ* breast cancer (27). Differences in geographic location of the studied population did not explain the differences in the role of the 936C>T SNP. Results of meta-analysis (Table 2) show that, except for 936 CC vs. (CT or TT), odds ratios for all other genotype comparisons were homogenous to be combined across studies. Despite a small number of studies that disagree with the others, the pooled analyses for these genotypes appear not to have found any significant associations with breast cancer risk.

VEGF SNPs in NSCLC

High VEGF mRNA or protein expression in tumor (35) or in serum have shown to be associated with advanced tumor stage (IIIB and IV) (36), shorter survival (37) and prognosis of NSCLC (38). VEGF mRNA and protein expression were found to be significantly higher in adenocarcinomas than in squamous-cell carcinomas (35). These observations suggest that SNPs in the *VEGF* gene may affect the prognosis or clinical outcome of NSCLC by altering the VEGF levels; studies evaluating this hypothesis are summarized in Table 3.

A small study on DNA samples extracted from tumor and normal lung tissues from 36 surgically resected patients, examined the effect of *VEGF* −2578C>A, −634G>C, −1154G>A, and 936C>T SNPs on tumor VEGF expression and vascular density (14). The −2578 CC, −634 GG and −1154 AA and GA genotypes were associated with low VEGF expression, while high VEGF levels were detected in samples carrying the −2578 CA, −634 GC and −1154 GG genotypes (14). The MVD in tumors bearing the −2578 CC and −634 GG genotypes was significantly lower compared to the −2578 CA and −634 CC+GC genotypes, respectively. *VEGF* −1154G>A and 936C>T were not related with tumor VEGF expression and MVD (14). A large case-control study on 1900 NSCLC Caucasian patients and 1458 healthy controls failed to find a correlation between germline *VEGF* −1498C>T, −634C>G and 936C>T SNPs taken singularly on risk of NSCLC after adjusting for age, gender, smoking status, pack-years of smoking, and years since smoking cessation (39). However, male patients bearing the −634CC+CG genotype and−1498T/−634G/936C haplotype respectively had higher and reduced risk of developing lung adenocarcinoma (39). The lack of association in female patients was unclear and was not explained by differences in smoking status between genders (39). Another study on 462 patients with early-stage NSCLC treated with surgical resection, examined the impact of germline 936C>T, −1498C>T, and −634G>C SNPs on survival (3). Carriers of −634C and 936T alleles had longer five-year overall survival than non-carriers, with respective survival percent of 61% vs. 51% and 67% vs. 54% (3). While considering combined effect of 936C>T and −634G>C SNPs, increased number of pooled variant alleles resulted in significantly longer survival, with adjusted HR for one, two and three variant allele (s) being 0.76, 0.61, and 0.61, respectively compared to the zero variant allele (3). The −1498C>T SNP had no association with overall survival in these patients (3).

In conclusion, male subjects carrying the −634C allele appear to be at increased risk of lung adenocarcinoma (39). Considering both males and females together, −634G>C, 936C>T and −1498C>T had no significant association with risk of disease (39) but variant allele for these SNPs predicted longer overall survival (3). The −1498T/−634G/936C haplotype may predict the risk of NSCLC. The −2578C>A, −1154G>A, and −634G>C SNPs were associated with VEGF expression and angiogenesis, but these observations were limited by small sample size (14). There were not enough studies (Table 3) to perform meta-analysis.

VEGF SNPs in colorectal cancer

Like other solid tumors, overexpression of *VEGF* mRNA and protein has shown to be associated with tumor progression and poor prognosis of colon carcinoma (40). VEGF has shown to be an important predictor of prognosis in cases of advanced CRC (41). High MVD and VEGF expression were shown to be related with poor relapse free and overall survival (42). Therefore, *VEGF* SNPs may play important role in determining the risk, prognosis and survival in colorectal cancer patients; studies evaluating this hypothesis are summarized in Table 4.

A study that analyzed *VEGF* SNPs in peripheral blood and their impact on prognosis in 125 patients with locally advanced colon cancer found that the *VEGF* 936CC genotype had a significantly shorter median time to relapse compared to CT or TT genotypes (2.6 vs. 11.1

years) (40). A case-control study on the *VEGF* 936C>T germline SNP in Korean colon cancer patients (n=262) and healthy controls (n=229) demonstrated that the frequency of T allele-bearing genotypes (CT+TT) was higher in cases than in controls. When stratified by gender and age, the frequency of CT+TT was significantly associated with increased risk for colon cancer in women and patients younger than 55 years, thus suggesting that the *VEGF* 936C>T SNP might be a genetic determinant for colon cancer in Korean patients (43). Another case-control study on the *VEGF* −2578C>A germline SNP was carried out in a cohort of 246 patients with colon cancer and 203 healthy controls and found that *VEGF* −2578C>A SNP had no association with susceptibility to colon cancer, although gender stratification indicated that the −2578CA and AA genotypes had a marginal protective effect for colon cancer in women. Therefore, the *VEGF* −2578C>A SNP, at least in Koreans, appears to have a borderline role in colon cancer risk (44). The analysis of two *VEGF* SNPs (−634G>C and 936C>T) in colorectal tissue from 465 patients and peripheral blood lymphocytes from 413 healthy controls demonstrated that the −634 GC and CC genotypes were associated with a decreased risk of cancer, indicating a dominant role of the C allele, whereas the 936TT genotype correlated with advanced stage, high serum levels of CA19-9, and higher histologic grades (45). Haplotype analysis showed that −634C/936C and −634G/936T were associated with decreased susceptibility of colorectal cancer (45). The *VEGF* −1498C>T, −634G>C and −7C>T SNPs, were determined in 36 Japanese patients and demonstrated that the −1498CC allele, but not the −634G>C and −7C>T SNPs, was predictive of poorly-differentiated tumors and thereby poor prognosis (46). A larger study on 445 patients with colorectal cancer examined the *VEGF* −2578C>A, −634G>C, and 936C>T SNPs and showed that the survival for patients with the −634GC or CC genotypes was better than for those with the −634GG genotype (47). Furthermore, the 936CT or TT genotypes were associated with a worse survival compared with the 936CC genotype and −2578C>A was not related with survival (47). Finally, in haplotype analysis, the −2578A/−634G/936T exhibited a significantly less favorable survival when compared with the wild type −2578C/−634G/936C haplotype (47). A case-control study examined *VEGF* 936C>T, −2578C>A, and −634G>C SNPs in 427 patients and 427 age-and sex-matched healthy control subjects and found no correlation between all these variants and tumor size, histological grading, positive regional lymph node metastases or tumor stage (48). Patients treated with cetuximab were genotyped to assess the possible predictive role of SNPs of *cyclin D1, cyclooxygenase 2, EGF, EGFR, IL-8* and *VEGF* genes in blood samples. While a significant association was found between *cyclin D1* 870A>G and the *EGF* 61A>G SNPs and survival, *VEGF* 936C>T showed no association (49). Finally, a study on RNA from 25 colorectal cancer tissues demonstrated the presence of *VEGF121, VEGF145, VEGF165* and *VEGF189* splice variants and an array of novel mutations within the conserved expression site of the gene. Five samples exhibited single nucleotide changes and in one sample a 2-nucleotide deletion was detected. In addition to this, a 1- and 2-base deletion with frameshift and protein truncation in exon 3 at positions +172 and +171/172 were shown, respectively, as well as a transition mutation in exon 3 at position +248 and two transition mutations in exon 4 at positions +398 and +403. The biologic and clinical impact of these variants were not demonstrated, however (50).

In conclusion, 936 T allele has shown to be associated with increased risk (43), advanced stage of disease (45), worse survival (47) and longer time to relapse (40), while other studies have shown no correlation with tumor size, grade and stage (48) and no relation with survival (49) in patients with colorectal cancer. The −634 C allele was predictive of decreased risk (45) and better survival (47), and was not related with tumor size, grade and stage (48). The *VEGF* −2578C>A SNP was not related with risk (44), survival (47), tumor size and stage (48), but −2578 CA and AA had borderline protective role in Korean women (44). The results of meta-analysis shown in Table 4 suggest that ORs for several genotypic comparisons were not homogeneous to be pooled across studies (p-value for test of homogeneity, <0.05). The 95% CI for all the genotypic comparison for which ORs were homogeneous and could be pooled

together included 1, suggesting no significant correlation of selected *VEGF* SNPs with risk of colorectal cancer.

VEGF SNPs in prostate cancer

Like some solid tumors, progressive growth and metastasis of prostate cancer is also dependent on angiogenesis. VEGF expression and vascular density were significantly higher in prostate cancer tissue compared to benign prostate hypertrophy (BPH) or normal prostate tissue (5, 51). Metastatic prostate cells had enhanced VEGF production and tumor vascularity than cells with low metastatic potential (52), suggesting the importance of VEGF in prostate cancer metastasis. Studies evaluating the role of *VEGF* SNPs in prostate cancer susceptibility, progression and metastasis are summarized in Table 5.

A case-control study on 247 cancer patients and 263 controls from the UK examined SNPs in five cytokines including *VEGF* −1154 G>A and demonstrated that −1154 AA genotype had reduced risk of prostate cancer (OR, 0.45) (53). Another small case-control study on the *VEGF* −1498C>T germline SNP in 96 prostate cancer patients and 119 controls of Taiwanese descent found that carriers of −1498T allele were at 2.3 times higher risk of prostate cancer than non-carriers (54). However, this SNP was not related with prognosis (i.e., clinical stage and pathological tumor grade) and response to hormonal therapy (54). A case-control study in Tunisian subjects evaluated the influence of germline −1154G>A, −634G>C, and 936C>T *VEGF* SNPs on risk and prognosis of prostate cancer in 101 patients and 100 age-matched healthy controls (55). Carriers of at least one −1154 A allele were at reduced risk (OR, 0.42) and lower susceptibility to high-grade (OR, 0.25) and advanced prostate cancer (OR, 0.37). The odds ratio for −1154 GA and AA genotypes were 0.46 and 0.27, respectively (55), agreeing with McCarron et al. (53) despite of differences in ethnicity. The *VEGF* −634 GC+CC genotypes were at increased risk of prostate cancer (OR, 1.95), and the −634GC and CC genotypes were related with high-grade tumors (OR, 3.83 and 4.89, respectively) (55). Haplotype analysis showed that −1154A/−634G had reduced disease risk (OR, 0.48), high-grade tumor (OR, 0.22) and advanced disease (OR, 0.45) compared to −1154G/−634G (55). No correlation was observed between 936C>T SNP and risk or aggressiveness of tumor (55). These results suggest that both *VEGF* −1154G>A and −634G>C SNPs may act as important genetic marker of risk and aggressiveness of prostate cancer in Tunisian subjects. A case-control study in 133 sporadic prostate cancer patients and 157 healthy controls of Turkish descent evaluated the germline −1498C>T SNP and found no relation with susceptibility to disease (56), which was contrary to the findings for Taiwanese subjects (54). Further, the −1498C>T SNP was not related with the TNM staging, Gleason scores and serum prostate specific antigen (PSA) levels of patients (56). The impact of the same germline *VEGF* −1498C>T SNP in Japanese population was examined in a case-control study enrolling 270 patients and 252 controls. Similar to the Turkish population (56), the −1498C>T SNP was not related with disease risk, tumor grade and stage, Gleason scores, VEGF expression, MVD and age at diagnosis (51). The rate of PSA relapse after radical prostatectomy was higher in patients with the 1498CC or CT genotype (51), but the TT genotype had significantly worse cancer-specific and overall survival (51).

Another study in Tunisian subjects determined the combined effect of four germline SNPs which are involved in the process of angiogenesis, i.e., *VEGF* −1154G>A, *VEGF* −634G>C, MMP9 −1562C>T and TSP1 −8831A>G, in predicting the risk of prostate cancer by enrolling 101 patients and 74 controls (57). The *VEGF* −1154AA, *VEGF* −634GG, MMP9 −1562CC and TSP1 −8831AA genotypes were considered as low-risk genotypes and were used as reference, and the remaining genotypes for each SNP were considered as high-risk genotypes (57). Patients with 1, 2, or 3 high-risk genotypes in *VEGF* −1154/*VEGF* −634/MMP9 group had increasingly high risk of prostate cancer (OR, 2.79, 4.57 and 7.11 respectively), compared

to zero high-risk genotype group (57). In the *VEGF*-1154/634/TSP1 group, patients carrying three high-risk genotypes had 6 times higher risk of prostate cancer (OR, 6.0) and were 20 times more prone to have high-grade tumors when compared with reference groups (OR, 20.75) (57). Overall, their findings show that the gene-gene interactions between high-risk angiogenic polymorphisms may increase the risk of prostate cancer and tumor aggressiveness (57). Finally, a case-control study in 702 patients and 702 age matched healthy controls from Austria analyzed 7 *VEGF* SNPs (-2578C>A, −2489C>T, −1498C>T, −634G>C, −7C>T, 936C>T and 1612G>A) and their haplotypes, and failed to find any relation between these SNPs and the risk of prostate cancer or VEGF plasma levels (58).

To conclude, studies from Austria, Japan, Taiwan, Tunisia, Turkey and the UK evaluated the importance of *VEGF* SNPs in prostate cancer. The *VEGF* −1154 A allele appears to predict the reduced risk (53,55), which was consistent among studies as shown by meta-analysis (Table 5), where the pooled OR (95%CI) for −1154 AA genotype was 0.40 (0.22–0.72). The *VEGF* −1154A allele also imparted protection against high-grade tumor and tumor aggressiveness (55). Except for Taiwanese subjects, where −1498T allele predicted high risk of prostate cancer (54), the −1498C>T SNP was not related with risk (51,56,58) and disease prognosis (i.e., with tumor grade, stage, Gleason scores, VEGF expression and MVD) (51,56,58). The −634C allele was predictive of increased risk and high tumor grades (55) in Tunisian subjects but had no relation with risk in Austrian subjects (58). The results of meta-analysis showed no significant association between any other *VEGF* polymorphism and prostate cancer risk or clinical outcome.

Conclusions

Many studies have investigated the role of *VEGF* polymorphisms as genetic determinant for susceptibility and outcome of breast, prostate, NSCL and colorectal cancer. Several polymorphisms have been reported within the promoter (-2578C>A, −2489C>T, −1498C>T and −1154G>A), 5'-UTR (-634G>C and −7C>T) and 3'-UTR (936C>T and 1612G>A) for the *VEGF* gene. The variant allele for −1154G>A and 936C>T results in lower VEGF expression, whereas the variant allele for −1498C>T and −7C>T results in increased concentrations of VEGF mRNA (15,59). The functional role of −2578C>A and −634G>C is not in agreement among studies, which report low or high VEGF production for variant alleles (15). Results of association studies of these polymorphisms with risk and outcome in breast, prostate, NSCL and colorectal cancer are also variable (Table 2–Table 5, Supplementary Table 6). For example the −936T allele has shown to impart protection against breast cancer (22, 26,32), but conversely increases the risk of colorectal cancer (43). Similarly, the −634C allele was predictive of higher risk of NSCLC (39) and prostate cancer (55), but predicts reduced risk for colon cancer (45) and has no relation with risk of breast cancer (24,26–29,31). The lack of consensus about the role of these SNPs could be because of their linkage with other unknown functional SNPs in the *VEGF* gene or unknown SNPs in other factors of the angiogenesis pathway. Perhaps, association with haplotypes will be more predictive than individual SNPs. More understanding about the functionality of these polymorphisms and their role in tumor biology is essential to discern the reasons for inconsistencies in the literature (60), which may also help in designing association studies in the future. Other possible reasons are differences in study population (i.e., ethnicity and tumor type), small sample size (insufficient power), and failure to correct for multiple testing when studying the correlation with more than one SNP, which is important to avoid chance correlations. However, likelihood of chance correlations should be assessed with consideration to the biological plausibility of any observed correlation (60).

Independently, several of these studies reported association between *VEGF* SNPs and risk of cancer (Table 2–Table 5); however, the findings from these studies appear to be inconsistent.

We performed meta-analysis to look at the combined results from studies addressing the same hypothesis. Except *VEGF* −1154G>A, no other *VEGF* SNP was found to be related with risk of studied tumors. The *VEGF* −1154A allele was at significantly reduced risk of prostate cancer (OR, 0.40; 95%CI, 0.22–0.72). Haplotype −2578A/−1154A/−634G and −1154A/−634G predicted reduced risk of breast (26) and prostate cancer (55).

The VEGF-mediated angiogenesis pathway involves a complex signaling network regulated by multiple factors and it is likely that final outcome is governed by interaction and cross-talk between these signaling molecules/receptors rather than the *VEGF* gene alone. In that case, a pathway approach, which includes combined analysis of variations in different genes in the angiogenesis pathway, may provide better predictive markers. For example carriers of three high risk genotypes i.e., *VEGF* −1154AA/*VEGF* −634GG/MMP9CC, had 7.11 times high risk of prostate cancer than *VEGF* −1154GG/*VEGF* −634CC/MMP9TT group (57). Genome-wide association (GWA) studies, performed to identify the candidate genes and chromosomal sites linked to cancer, showed that locus including the VEGF gene was not associated with risk of breast, NSCLC, colon and prostate cancer (61–71). However, our meta-analysis suggests that VEGF SNPs predict the risk of prostate cancer, indicating their role in vascular growth, at the minimal in prostate cancer, although they may not be as strongly predictive (i.e. penetrant) as other alleles in the genome. It should also be noted that there are other limitations to GWA approach, such as multiple testing in small population and low-density genotyping (72,73), which may limit the identification of true susceptibility genes.

Most of the published studies assessing the role of *VEGF* polymorphisms compare the cases with controls to identify the disease markers. There is critical need to conduct more studies evaluating the relation of genotype with drug concentrations and response. These studies are equally important and helpful for identification of genetic markers to guide the choice of treatment and supportive care specific to each individual.

Abbreviations

VEGF, vascular endothelial growth factor; SNP, single nucleotide polymorphism; NSCLC, non-small cell lung cancer; CRC, colorectal cancer; MVD, micro-vessel density; OR, odds-ratio.

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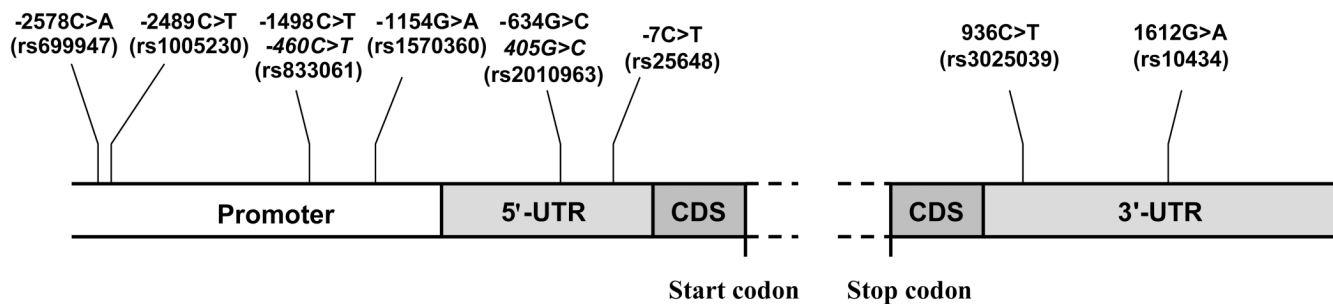
Fig. 1.
Structure of VEGF gene and position of VEGF SNPs relative to translation start site. *Italic positions* indicate alternate names. Dashed lines indicate the region consisting of coding sequence (CDS) and seven introns. (adapted from reference (29)). UTR: Untranslated region.

Table 1

Frequencies of selected VEGF polymorphisms in control subjects

Polymorphism	Ethnicity	Controls	Variants N (% Genotype Frequency)			Allele Frequency		Ref.
			CC	CT	TT	p	q	
936 C>T								
(rs3025039)	Austrian Caucasian	500	353 (70.6)	137 (27.4)	10 (2.0)			(22)
	Polish	422	297 (70.4)	114 (27.0)	11 (2.6)			(24)
	German	163	128 (78.5)	31 (19.0)	4 (2.5)			(24)
	Swedish	934	720 (77.1)	203 (21.7)	11 (1.2)			(24)
	Chinese	1195	793 (66.4)	351 (29.4)	51 (4.3)			(24)
	UK Caucasian	708	531 (75.0)	165 (23.3)	12 (1.7)			(28)
	98% Caucasian	479	353 (73.7)	118 (24.6)	8 (1.7)			(27)
	NR	60	57 (95.0)	3 (5.0)	0 (0.0)			(30)
	Polish Caucasian	290	202 (69.7)	81 (27.9)	7 (2.4)			(32)
	Caucasian	1458	1125 (77.2)	308 (21.1)	25 (1.7)			(39)
	Korean	229	169 (73.8)	57 (24.9)	3 (1.3)			(43)
	Korean	413	252 (61.0)	149 (36.1)	12 (2.9)			(45)
	Austrian	427	308 (72.1)	108 (25.3)	11 (2.6)			(48)
	Tunisian	100	72 (72.0)	27 (27.0)	1 (1.0)			(55)
	Austrian	804				0.85	0.15	(29)
			GG	GA	AA			
−1154G->A								
(rs1570360)	NR	263	120 (45.6)	109 (41.4)	34 (12.9)			(23)
	Polish	423	199 (47.0)	178 (42.1)	46 (10.9)			(24)
	German	163	76 (46.6)	69 (42.3)	18 (11.0)			(24)
	98% Caucasian	492	208 (42.3)	227 (46.1)	57 (11.6)			(27)
	UK Caucasian	263	120 (45.6)	109 (41.4)	34 (12.9)			(53)
	Tunisian	100	36 (36.0)	50 (50.0)	14 (14.0)			(55)
			GG	GC	CC			
−634 G>C								
(rs2010963)	Swedish	941	492 (52.3)	367 (39.0)	82 (8.7)			(24)
	98% Caucasian	500	232 (46.4)	221 (44.2)	47 (9.4)			(27)

Polymorphism	Ethnicity	Controls	Variants N (% Genotype Frequency)			Allele Frequency		Ref.
						p	q	
			CC	**CA**	**AA**			
	Korean	413	106 (25.7)	223 (54.0)	84 (20.3)			(45)
	Austrian	430	192 (44.7)	195 (45.4)	43 (10.0)			(48)
	Tunisian	100	44 (44.0)	46 (46.0)	10 (10.0)			(55)
	Chinese	1198	418 (34.9)	598 (49.9)	182 (15.2)			(26)
	UK Caucasian	498	209 (42)	225 (45.2)	64 (12.9)			(28)
	Caucasian	1458	650 (44.6)	644 (44.2)	164 (11.3)			(39)
	Austrian	804				0.66	0.34	(29)
−2578 C>A (rs699947)			**CC**	**CA**	**AA**			
	Polish	423	106 (25.1)	207 (48.9)	110 (26.0)			(24)
	German	162	50 (30.9)	72 (44.4)	40 (24.7)			(24)
	Swedish	940	257 (27.3)	451 (48.0)	232 (24.7)			(24)
	98% Caucasian	495	129 (26.1)	236 (47.7)	130 (26.3)			(27)
	Korean	203	106 (52.2)	82 (40.1)	15 (7.4)			(4)
	Austrian	427	85 (19.9)	238 (55.7)	104 (24.4)			(48)
	Austrian	804				0.53	0.47	(29)
−1498 C>T (rs833061)			**CC**	**CT**	**TT**			
	Chinese	1222	78 (6.4)	479 (39.2)	665 (54.4)			(26)
	UK Caucasian	498	106 (21.3)	251 (50.4)	141 (28.3)			(28)
	Caucasian	1458	342 (23.5)	694 (47.6)	422 (28.9)			(39)
	Taiwanese	119	4 (3.4)	72 (60.5)	43 (36.1)			(54)
	Turkish	157	50 (31.9)	94 (59.9)	13 (8.3)			(56)
	Japanese	252	23 (9.1)	97 (38.5)	132 (52.4)			(51)
	Austrian	804				0.54	0.46	(29)
−7 C>T (rs25648)			**CC**	**CT**	**TT**			
	UK Caucasian	493	332 (67.3)	151 (30.7)	10 (2.0)			(28)
	Austrian	804				0.84	0.16	(29)

Polymorphism	Ethnicity	Controls	Variants			Allele Frequency		Ref.
			N (% Genotype Frequency)			p	q	
			CC	CT	TT			
−2489C>T								
(rs1005230)	Austrian	804				0.53	0.47	(29)
			GG	GA	AA			
1612G>A								
(rs10434)	Austrian	804				0.54	0.46	(29)

NR: not reported

Table 2

Summary of case-control studies assessing the VEGF polymorphisms in relation to risk of breast cancer

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with respect to VEGF Genotype	OR	OR Adjusted (Yes/No)	L95%CI	U95%CI	Ref
936 C>T[1]	Austrian Caucasian	500/500	CC vs. (CT or TT)*	Yes (decreases risk)	0.51	No	0.38	0.7	(22)
			TT vs. CC*	No	0.77	No	0.29	2.03	
	Polish	412/422	CT vs. CC	No	0.87	No	0.63	1.21	(24)
			TT vs. CC	No	1.27	No	0.53	3.05	
			CC vs. (CT or TT)*	No	0.91	No	0.67	1.23	
	German	153/163	CT vs. CC	No	1.07	No	0.59	1.93	(24)
			TT vs. CC	No	0.53	No	0.07	3.46	
			CC vs. (CT or TT)*	No	1.01	No	0.58	1.75	
	Swedish	924/934	CT vs. CC	No	1.02	No	0.81	1.28	(24)
			TT vs. CC	No	1.11	No	0.45	2.71	
			CC vs. (CT or TT)*	No	1.03	No	0.82	1.28	
	Chinese	1109/1195	CT vs. CC	No	1.01	No	0.85	1.21	(26)
			TT vs. CC	Yes (decreases risk)	0.65	No	0.41	1.02	
			CC vs. (CT or TT)*	No	0.97	No	0.81	1.15	
	Caucasian (98%)	488/479	CC vs. (CT or TT)	No	1.02	Yes	0.76	1.36	(27)
	UK Caucasian	848/708	TT vs. CC*	No	1.42	No	0.67	3.07	(28)
			CC vs. (CT or TT)*	No	1.08	No	0.85	1.36	
	Polish Caucasian	319/290	TT vs. CC*	No	0.82	No	0.26	2.6	(32)
			CC vs. (CT or TT)	Yes (decreases risk)	0.69	No	0.48	0.99	
	NR	60/60	CC vs. (CT or TT)*	Yes (increases risk)	6.91	Yes	1.84	32	(30)
−2578 C>A[2]	Polish	411/423	CA vs. CC	No	0.96	No	0.68	1.36	(24)
			AA vs. CC	No	1.04	No	0.7	1.54	
			AA vs. (CA or CC)*	No	1.07	No	0.78	1.46	
	German	153/162	CA vs. CC	No	1.18	No	0.68	2.06	(24)
			AA vs. CC	No	0.97	No	0.5	1.86	

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with respect to VEGF Genotype	OR	OR Adjusted (Yes/No)	L95%CI	U95%CI	Ref
			AA vs. (CA or CC)*	No	0.87	No	0.51	1.49	
	Swedish	939/940	CA vs. CC	No	0.99	No	0.79	1.24	(24)
			AA vs. CC	No	1	No	0.77	1.29	
			AA vs. (CA or CC)*	No	1	No	0.81	1.24	
	98% Caucasian	498/495	CA vs. AA	No	1.18	Yes	0.86	1.61	(27)
			AA vs. CC†	No	0.8	Yes	0.56	1.14	
	89% Cau + 7% AA	NR (total 656)	AA vs. (CA or CC)	Yes (increases risk)	1.99	Yes	1.06	3.74	(31)
1154 G>A[3]	NR	134/263	AA vs. GG*	No	0.61	No	0.27	1.3	(23)
	Polish	411/423	GA vs. GG	No	1.22	No	0.91	1.65	(24)
			AA vs. GG	No	1.23	No	0.76	1.97	
	German	159/163	GA vs. GG	No	0.76	No	0.46	1.25	(24)
			AA vs. GG	No	0.85	No	0.39	1.89	
	98% Caucasian	495/492	GA vs. AA	No	1.01	Yes	0.66	1.55	(27)
			AA vs. GG†	No	0.78	Yes	0.51	1.19	
634 G>C[4]	Chinese	1095/1198	CG vs. CC	No	0.81	No	0.64	1.02	(26)
			CC vs. GG†	No	1.12	No	0.88	1.44	
	UK Caucasian	490/498	CG vs. CC*	No	1.03	No	0.68	1.56	(28)
			CC vs. GG*	No	0.82	No	0.54	1.26	
			GC vs. GG*	No	0.85	No	0.65	1.12	
	Swedish	936/941	GC vs. GG	No	1	No	0.82	1.21	(24)
			CC vs. GG	No	1.05	No	0.74	1.47	
			CG vs. CC*	No	0.95	No	0.68	1.34	
	98% Caucasian	495/500	GC vs. GG	No	1.05	Yes	0.8	1.37	(27)
			CC vs. GG	No	1.18	Yes	0.76	1.83	

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with respect to VEGF Genotype	OR	OR Adjusted (Yes/No)	L95%CI	U95%CI	Ref
−1498 C>T[5]	Chinese	1123/1222	CT vs. TT	No	0.94	No	0.79	1.12	(26)
			CC vs. TT	No	1.23	No	0.89	1.7	
			CC vs. (CT or TT)*	No	1.26	No	0.92	1.73	
	UK Caucasian	493/498	CT vs. TT*	No	1.04	No	0.77	1.4	(28)
			CC vs. TT*	No	1.1	No	0.77	1.59	
			CC vs. (CT or TT)*	No	1.07	No	0.8	1.45	
	89% Cau + 7% AA	NR (total 656)	CC vs. (CT or TT)	Yes (increases risk)	2.01	Yes	1.08	3.76	(31)
1498T/−634G/936T	Chinese		Ref: −1498T/−634C/936C	Yes (decreases risk)	0.67	No	0.43	1.04	(26)
1498T/−634G/936C				No	0.97	No	0.84	1.11	
1498C/−634G/936C				No	1.02	No	0.86	1.21	
1498C/−634G/936T				No	0.96	No	0.78	1.18	
1498T/−634C/936T				No	0.98	No	0.76	1.25	
−2578A/−1154A/−634G	98% Caucasian		1 HC vs. 0	Yes (decreases risk)	0.69	Yes	0.51	0.92	(27)
			2 HC vs. 0	Yes (decreases risk)	0.62	Yes	0.38	1	
−2578A/−1154G/−634G			1 HC vs. 0	No	0.97	Yes	0.71	1.33	
			2 HC vs. 0	No	1.71	Yes	0.7	4.15	
−2578C/−1154G/−634G			1 HC vs. 0	No	1.27	Yes	0.94	1.72	
			2 HC vs. 0	No	1.68	Yes	0.78	3.61	
−2578C/−1154G/−634C			1 HC vs. 0	No	1.07	Yes	0.8	1.43	

*
Calculated odds ratio

[†]
OR inverted from original publication. HC-Haplotype copy numbers

[1]
Test for homogeneity of odds ratio: Pooled OR (95%CI); CT vs. CC, p=0.86, 0.99 (0.88–1.13), TT vs. CC, p=0.61, 0.86 (0.64–1.16), CC vs. (CT or TT), p=0.0010, 0.91 (0.76–1.10)

[2]
Test for homogeneity of odds ratio: Pooled OR (95%CI); CA vs. CC, p=0.82, 1.00 (0.84–1.20), AA vs. CC, p=0.73, 0.95 (0.80–1.13), AA vs. (CA or CC), p=0.20, 1.08 (0.86–1.35)

[3] Test for homogeneity of odds ratio: Pooled OR (95%CI); AA vs. GG, p=0.36, 0.89 (0.67–1.18), GA vs. GG, p=0.11, 1.01 (0.64–1.59)

[4] Test for homogeneity of odds ratio: Pooled OR (95%CI); GC vs. GG, p=0.52, 0.97 (0.85–1.11), GC vs. CC, p=0.52, 0.88 (0.74–1.05), CC vs. GG, p=0.62, 1.06 (0.89–1.25)

[5] Test for homogeneity of odds ratio: Pooled OR (95%CI); CT vs. TT, p=0.57, 0.96 (0.83–1.12), CC vs. TT, p=0.66, 1.17 (0.92–1.49), CC vs. (CT or TT), p=0.21, 1.23 (1.00–1.51)

Table 3

Summary of case-control studies assessing the VEGF polymorphisms in relation to risk of NSCLC

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with respect to VEGF Genotype	Gender	OR	L95%CI	U95%CI	Ref
936 C>T	Caucasian	1900/1458	(CT or TT) vs. CC	No	All	1.05	0.87	1.27	(39)
				No	Male	1.13	0.87	1.5	
				No	Female	0.99	0.27	1.29	
−1498 C>T	Caucasian	1900/1458	(CT or CC) vs. TT	No	All	1.16	0.96	1.4	(39)
				No	Male	1.07	0.81	1.4	
				No	Female	1.27	0.98	1.65	
−634 G>C	Caucasian	1900/1458	(CG or CC) vs. GG	No	All	1.14	0.96	1.36	(39)
				Yes (increases risk, lung adenocarcinoma)	Male	1.27	0.99	1.63	
				No	Female	1.04	0.82	1.32	
−1498T/−634C/936C	Caucasian	1900/1458	**Ref** −1498C/−634G/936C	No	All	1.02	0.88	1.17	(39)
				No	Male	1.15	0.94	1.42	
				No	Female	0.91	0.74	1.11	
−1498T/−634G/936C	Caucasian	1900/1458	**Ref** −1498C/−634G/936C	No	All	0.88	0.75	1.04	(39)
				Yes (decreases risk, lung adenocarcinoma)	Male	0.81	0.63	1.02	
				No	Female	0.93	0.74	1.17	
−1498C/−634G/936T	Caucasian	1900/1458	**Ref** −1498C/−634G/936C	No	All	0.98	0.74	1.31	(39)
				No	Male	0.9	0.59	1.36	
				No	Female	1.04	0.69	1.55	

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with respect to VEGF Genotype	Gender	OR	L95%CI	U95%CI	Ref
−1498T/−634C/936T	Caucasian	1900/1458	**Ref** −1498C/−634G/936C	No	All	1.14	0.84	1.55	(39)
				No	Male	1.15	0.72	1.85	
				No	Female	1.04	0.69	1.57	

Table 4

Summary of case-control studies assessing the VEGF polymorphisms in relation to risk of Colon cancer

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with Respect to VEGF Genotype	OR	L95%CI	U95%CI	Ref
936C>T[1]	Korean	262/229	CT vs. CC	No	1.45	0.97	2.16	(43)
			TT vs. CC	No	2.98	0.79	11.21	
			(CT or TT) vs. CC	Yes (increases risk)	1.52	1.03	2.25	
	NR	465/413	CT vs. CC	No	0.89	0.67	1.18	(45)
			TT vs. CC	No	1.19	0.5	2.35	
			(CT or TT) vs. CC	No	0.92	0.7	1.21	
			T vs. C	No	0.96	0.76	1.21	
	Austrian	427/427	CT vs. CC*	No	0.76	0.55	1.05	(48)
			TT vs. CC*	No	0.68	0.27	1.7	
			(CT or TT) vs. CC*	No	0.75	0.55	1.02	
−2578C>A[2]	Korean	246/203	CA vs. CC	No	0.72	0.49	1.07	(44)
			AA vs. CC	No	0.66	0.31	1.43	
			(CA or AA) vs. CC	No	0.71	0.49	1.04	
	Austrian	433/427	CA vs. CC*	No	1	0.7	1.43	(48)
			AA vs. CC*	No	1.31	0.88	1.95	
			(CA or AA) vs. CC*	No	1.1	0.78	1.54	
−634G>C[3]	NR	465/413	GC vs. GG	Yes (decreases risk)	0.55	0.41	0.75	(45)
			CC vs. GG	No	0.81	0.55	1.18	
			(GC or CC) vs. GG	Yes (decreases risk)	0.62	0.47	0.83	
			C vs. G	No	0.86	0.71	1.04	
	Austrian	432/430	GC vs. GG*	No	0.98	0.74	1.3	(48)
			CC vs. GG*	No	1.09	0.69	1.72	
			(GC or CC) vs. GG*	No	1	0.76	1.31	
−634C/936C	NR		**Ref**: −634G/936C	Yes (decreases risk)	0.53	0.43	0.66	(45)

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with Respect to VEGF Genotype	OR	L95%CI	U95%CI	Ref
−634G/936T	NR		**Ref**: −634G/936C	Yes (decreases risk)	0.56	0.42	0.74	Ref

*
Calculated odds ratio. NR-Not Reported

[1] Test for homogeneity of odds ratio: Pooled OR (95%CI); CT vs. CC, p=0.042, 0.98 (0.70–1.37), TT vs. CC, p=0.20, 1.17 (0.57–2.39), (CT or TT) vs. CC, p=0.019, 1.00 (0.69–1.44)

[2] Test for homogeneity of odds ratio: Pooled OR (95%CI); CA vs. CC, p=0.22, 0.86 (0.62–1.19), AA vs. CC, p=0.12, 1.01 (0.53–1.93), (CA or AA) vs. CC, p=0.095, 0.89 (0.58–1.36)

[3] Test for homogeneity of odds ratio: Pooled OR (95%CI); GC vs. GG, p=0.0070, 0.74 (0.42–1.30), CC vs. GG, p=0.33, 0.91 (0.68–1.22), (GC or CC) vs. GG, p=0.018, 0.79 (0.49–1.26)

Table 5

Summary of case-control studies assessing the VEGF polymorphisms in relation to risk of prostate cancer

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with Respect to VEGF Genotype	OR	L95%CI	U95%CI	OR Adjusted	L95%CI	U95%CI	Ref
1498C>T[1]	Taiwanese	96/119	CT vs. CC	No	0.4	0.1	1.9	0.3	0.1	1.5	(54)
			TT vs. CC	No	1.4	0.3	5.9	1.1	0.2	4.9	
			T vs. C	Yes (increases risk)	2.3	1.4	3.8	2.2	1.3	3.8	
	Turkish	133/157	CT vs. CC	No	1.44	0.84	2.42				(56)
			TT vs. CC	No	1.28	0.51	3.2				
			(CT or TT) vs. CC	No	1.42	0.84	2.37				
	Japanese	270/252	CT vs. TT	No				0.98	0.68	1.4	(51)
			TT vs. CC[†]	No				1.02	0.97	1.39	
			(CT or TT) vs. CC*	No	1.03	0.57	1.88				
−1154G>A[2]	Tunisian	101/100	GA vs. GG	Yes (decreases risk)	0.46	0.24	0.87				(55)
			AA vs. GG	Yes (decreases risk)	0.27	0.08	0.83				
			(GA or AA) vs. GG	Yes (decreases risk)	0.42	0.23	0.76				
			(GA or GG) vs. AA*	No	0.39	0.12	1.14				
	UK Caucasian	238/263	GA vs. GG*	No	1.05	0.72	1.55				(53)
			(GA or GG) vs. AA	Yes (decreases risk)	0.45	0.24	0.86				
			AA vs. GG*	Yes (decreases risk)	0.46	0.22	0.93				
			(GA or AA) vs. GG*	No	0.91	0.63	1.32				
−634G>C	Tunisian	101/100	GC vs. GG	No	1.88	0.98	3.62				(55)
			CC vs. GG	No	2.28	0.82	6.38				
			(GC or CC) vs. GG	Yes (increases risk)	1.95	1.04	3.65				
	Austrian	702/702	CC vs. GG*	No	1.08	0.86	1.35				(58)
936C>T	Tunisian	101/100	CT vs. CC	No	0.68	0.33	1.37				(55)

Polymorphism	Ethnicity	Case/Control	Genotype	Association of Cancer Risk with Respect to VEGF Genotype	OR	L95%CI	U95%CI	OR Adjusted	L95%CI	U95%CI	Ref
			TT vs. CC	No	1.82	0.13	51.93				
			(CT or TT) vs. CC	No	0.72	0.36	1.43				
	Austrian	702/702	TT vs. CC*	No	1.16	0.91	1.48				(58)
−2578C>A	Austrian	702/702	CC vs. AA*	No	0.96	0.75	1.24				(58)
−2489C>T	Austrian	702/702	CC vs. TT*	No	0.97	0.75	1.25				(58)
−1498C>T	Austrian	702/702	CC vs. TT*	No	1	0.77	1.28				(58)
−7C>T	Austrian	702/702	CC vs. TT*	No	1.09	0.86	1.39				(58)
1612G>A	Austrian	702/702	GG vs. AA*	No	0.95	0.74	1.23				(58)
−1154G/−634C	Tunisian	101/100	**Ref:** −1154G/−634G	No	1.12	0.67	1.88				(55)
−1154A/−634G	Tunisian			Yes (decreases risk)	0.48	0.28	0.84				
−1154A/−634C	Tunisian			No	0.91	0.28	2.99				

*
Calculated odds ratio

†
OR inverted from original publication

1
Test for homogeneity of odds ratio: Pooled OR (95%CI); CT vs. CC, p=0.069, 0.80 (0.18–3.54), TT vs. CC, p=0.90, 1.05 (0.78–1.40), (CT or TT) vs. CC, p=0.42, 1.24 (0.84–1.83)

2
Test for homogeneity of odds ratio: Pooled OR (95%CI); GA vs. GG, p=0.029, 0.72 (0.32–1.63), AA vs. GG, p=0.41, **0.40 (0.22–0.72)**, (GA or AA) vs. GG, p=0.030, 0.64 (0.30–1.38)