Influence of polymorphisms in the vascular endothelial growth factor gene on allograft rejection after kidney transplantation: a meta-analysis [version 1; peer review: 2 approved]

Thanee Eiamsitrakoon¹, Phuntila Tharabenchasin¹, Noel Pabalan¹, Hamdi Jarjanazi², Adis Tasanarong³

¹Chulabhorn International College of Medicine, Thammasat University, Rangsit, Pathumthani, 12121, Thailand
²Environmental Monitoring and Reporting Branch, Ontario Ministry of the Environment, Conservation and Parks, Toronto, Ontario, M5T 3L9, Canada
³Nephrology Unit, Faculty of Medicine, Thammasat University, Rangsit, Pathumthani, 12121, Thailand

Abstract
Background: Reported associations of allograft rejection in kidney transplant patients with VEGF single nucleotide polymorphisms (SNPs) have been inconsistent between studies, which prompted a meta-analysis to obtain more precise estimates.

Methods: Using the PICO elements, kidney transplant patients (P) were compared by genotype data between rejectors (I) and non-rejectors (C) in order to determine the risk of allograft rejection (O) attributed to the VEGF SNPs. Literature search of four databases yielded seven articles. To calculate risks for allograft rejection, four SNPs were examined. Using the allele-genotype model we compared the variant (var) with the wild-type (wt) and heterozygous (var-wt) alleles. Meta-analysis treatments included outlier and subgroup analyses, the latter was based on ethnicity (Indians/Caucasians) and rejection type (acute/chronic). Multiple comparisons were corrected with the Bonferroni test.

Results: Five highly significant outcomes (P< 0.01) survived Bonferroni correction, one of which showed reduced risk for the var allele (OR 0.61, 95% CI 0.45-0.82). The remaining four indicated increased risk for the wt allele where the chronic rejection (OR 2.10, 95% CI 1.36-3.24) and Indian (OR 1.44, 95% CI 1.13-1.84) subgroups were accorded susceptibility status.

Conclusions: Risk associations for renal allograft rejection were increased and reduced on account of the wt and var alleles, respectively. These findings could render the VEGF polymorphisms useful in the clinical genetics of kidney transplantation.

Open Peer Review
Approval Status

| | 1 | 2 |
|---|---|---|
| version 1 | ✓ | ✓ |

1. Ileana Constantinescu, Fundeni Clinical Institute, Bucharest, Romania
2. Ion Maruntelu, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
3. Iman Karimzadeh, Shiraz University of Medical Sciences, Shiraz, Iran

Any reports and responses or comments on the article can be found at the end of the article.
Keywords
VEGF polymorphisms, allograft, renal, kidney transplantation, meta-analysis

Corresponding author: Noel Pabalan (noelpabalan@mail.com)

Author roles: Eiamsitrakoon T: Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Tharabenjasin P: Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Pabalan N: Conceptualization, Formal Analysis, Methodology, Resources, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Jarjanazi H: Methodology, Resources, Validation; Tasanarong A: Resources, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2021 Eiamsitrakoon T et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Eiamsitrakoon T, Tharabenjasin P, Pabalan N et al. Influence of polymorphisms in the vascular endothelial growth factor gene on allograft rejection after kidney transplantation: a meta-analysis [version 1; peer review: 2 approved]
F1000Research 2021, 10:90 https://doi.org/10.12688/f1000research.27800.1

First published: 10 Feb 2021, 10:90 https://doi.org/10.12688/f1000research.27800.1
Abbreviations
A, adenine; AR, acute rejection; C, cytosine; CA or C/A, cytosine/adename; CEU, European population; CI, confidence interval; CR, chronic rejection; CRAD, chronic renal allograft dysfunction; C/T, cytosine/thymine; du, duplicate; G, guanine; GIH, Gujarati Indian population; GG or G/G, guanine/guanine; het, heterozygous genotype; HW, Hardy-Weinberg Compliant HWE, Hardy-Weinberg Equilibrium; F, measure of variability; ITU, Telugu Indian population; KT, kidney transplantation; LD, linkage disequilibrium; n, number of studies; NRJ, non-rejection; OR, odds ratio; P, P-value for association; P_het, P-value for heterogeneity; [R], reference of studies; RJ, rejection; SNP, single nucleotide polymorphism; T, thymine; var, variant allele or genotype; VEGF, vascular endothelial growth factor gene; VEGF, vascular endothelial growth factor protein; wt, wild-type allele or genotype

Introduction
Chronic kidney disease is a longstanding global health problem with substantial effects on morbidity and mortality.

Even with medical intervention, the likely endpoints in the progression of this disease are end-stage renal disease and kidney failure. In such cases, kidney transplantation (KT) is the current best available therapeutic option.

Success of the transplanted organ or an allograft in the recipient is limited by graft rejection, which is characterized by inflammatory responses toward the graft tissue resulting in structural and functional impairments leading to allograft dysfunction.

Allograft rejection can be categorized largely into acute rejection (AR) which occurs days/weeks up until three months post-KT, or chronic rejection (CR) which is seen as progressive loss of graft function after three months post-KT.

Key factors that contribute to allograft rejection may involve cytokines that are secreted by immune cells and antibodies against graft antigens.

Cytokines have been recognized as potent immunomodulatory biomolecules that mediate physiological and pathological immune responses. These molecules determine the magnitude of alloimmune responses after transplantation, which influence graft survival.

Differences in genetic background of transplant recipients are, in part, the cause of varying immune responses towards grafts.

Recognizing these genetic differences and their effects on the immune response may help establish individualized immunosuppressive regimens that can improve allograft outcome. This is accomplished by identifying the alleles that may increase risk or confer protection for immune-mediated complications after KT.

Single nucleotide polymorphisms (SNPs) in the cytokine genes may impact graft survival by altering transcriptional activities and levels of gene expression which lead to variations in cytokine production.

Of the cytokine factors related to immune-mediated renal graft injury, the vascular endothelial growth factor (VEGF) is of potential use as a post-transplantation biomarker. As mediator of vascular formation, VEGF promotes endothelial cell proliferation, differentiation and survival. It also mediates endothelium-dependent vasodilation and maintains vascular permeability. Dysregulations of VEGF expression are evident in many renal abnormalities. This suggests a possible pathologic role of this protein in renal diseases including graft injury.

Studies of allograft tissues from rat KT models (in both AR and CR events) and human KT recipients with AR showed increased VEGF expression in renal tubules and interstitium. This suggests involvement of this gene/protein in the pathogenesis of allograft rejection. Various SNPs in the VEGF gene have been identified and reported to be associated either with low or high VEGF protein production.

One of the common VEGF SNPs, a cytosine (C) to adenine (A) polymorphism at position 2578 within the promoter region (-2578 C/A), was found to be associated with VEGF expression and allograft rejection. The CC genotype was associated with high VEGF production but varied in its effects on renal allograft outcomes with reduced and increased rejection risks across the studies.

Our study aims to provide better understanding of the genetic role of VEGF SNPs on post-KT allograft outcome in terms of risk for allograft rejection among recipients, which might guide potential future directions in transplant genetics.

To obtain less ambiguous, clearer estimates of the VEGF role in this investigation, we apply meta-analysis techniques (i.e. outlier treatment) in order to strengthen the evidence.

Methods
Selection of studies
We searched for association studies on 13 February 2020, the start date for this meta-analysis. Four strings of search terms were used that included combinations of “vascular endothelial growth factor”, “VEGF”, “polymorphism”, “cytokine”, “renal”, “transplant”, “allograft”, and “kidney transplantation” as medical subject heading and text in MEDLINE using PubMed, Google Scholar, Science Direct and Mednar, unrestricted by language. Details of the search strategies for each of these four databases are shown in Table S1 (Extended data).

References cited in the retrieved articles were also hand-screened to identify additional eligible studies. In case of duplicate articles, we selected the one with a later date of publication.

The following PICO elements were applied in the meta-analysis:

(i) Population: renal allograft patients; (ii) Intervention: VEGF gene polymorphisms; (iii) Comparators: rejectors (RJ) versus non-rejectors (NRJ); and (iv) Outcome: allograft rejection post-KT.

Inclusion criteria were: (i) case–control design evaluating the association between VEGF SNPs and risk of allograft rejection; (ii) available VEGF genotype frequencies in the presence and absence of allograft rejection and (iii) sufficient genotype frequency data to enable calculation of the odds ratios (ORs) and 95% confidence intervals (CIs).
**SNP groupings**

We examined four SNPs (Table 1; Extended data: S2 Table35). Observed phenotypic associations have been attributed to the proximity of SNPs in the VEGF gene16–28, termed linkage disequilibrium (LD). LD is the correlation between alleles located near each other29 and is measured in terms of D’ and r2 with a value of 1 indicating complete LD30,31. LD values were based on the European (CEU), and the Indian populations (Gujarati: GIH and Telugu: ITU) from LDLink. Complete LD between rs699947 (-2578C/A) and rs144854329 (-2549 insertion/deletion) merited combination, labeled VEGF1. -1154G/A (rs1570360), and 938C/T (rs3025039) were not in complete LD, thus analyzed separately, noted as VEGF2 and VEGF3, respectively (Table 2).

**Data extraction and Hardy-Weinberg Equilibrium (HWE)**

Two investigators (TE and NP) independently extracted data and arrived at a consensus. Authors of the component articles were contacted in cases of missing data. The following information were obtained from each publication: first author’s name, year of the study, country of origin, ethnicity, age of the subjects, comparators, VEGF SNPs (rs number), including transplant outcome in term of type of allograft rejection and values needed to tally the Clark-Baudouin score (Table 1). Sample sizes as well as genotype data in RJ and NRJ were also extracted along with calculated outcomes of the minor allele frequency. HWE was assessed using the application in https://ihg.gsf.de/cgi-bin/hw/hwa1.pl. HWE was reported as P-values of the controls from the Pearson’s goodness-of-fit χ2-square test.

**Statistical power and quality of the studies**

Using the G*Power program35, we evaluated statistical power. Assuming an OR of 1.5 at a genotypic risk of α = 0.05, power was considered adequate at ≥80%. Methodological quality of the included studies was assessed with the Clark-Baudouin scale30. In this scale, scores of <5, 5–6 and ≥7 represent low, moderate and high quality, respectively.

**Meta-analysis**

Given the hypothesis of association between VEGF SNPs and risk of allograft rejection following KT, we estimated the ORs with 95% CIs for each study by comparing RJ with NRJ among transplant recipients. Table 2 shows the frequencies of the variant (var) and wild-type alleles, as well as wt-var or heterozygous genotype (het). Ununiformity of the variant (var) allele in VEGF1 and VEGF2 warranted the use of the allele-genotype model for VEGF1 and VEGF2. On the other hand, the var alleles in VEGF3 (rs3025039) were uniform (all < 0.50), so the standard genetic models were suitable: (i) homozygous: var–var and wt–wt genotypes compared with wt–wt; (ii) recessive: var–var versus het + wt–wt; (iii) dominant: var–var + het versus wt–wt; and (iv) codominant: var versus wt. Using raw data for frequencies, study specific risks (ORs) of allograft rejection were estimated and pooled ORs were calculated by comparing the effects on the same baseline. Multiple comparisons were corrected with the Bonferroni test. Subgrouping was based on ethnicity (Indians/Caucasians) and type of rejection (AR/CR). High significance (P < 0.0001) indicated strong evidence for association.

Heterogeneity in meta-analysis31 was addressed with the following: (i) its presence warranted use of the random-effects model35, otherwise fixed-effects model35 was used; (ii) estimated with the χ2-based Q test35; (iii) quantified with the F statistic35; and (iv) sources were outlier treated. Outlier treatment divided the comparisons into pre-outlier and post-outlier.

**Table 1. Characteristics of the included studies in VEGF meta-analysis.**

| First author       | [R] | Year | Country | Ethnicity | Age (y) mean ± SD | Comparisons (/: versus) | VEGF polymorphisms (KT outcome) n | Clark-Baudouin score |
|--------------------|-----|------|---------|-----------|-------------------|------------------------|-----------------------------------|----------------------|
| Mittal             | 39  | 2011 | India   | Indian    | 36.1 ± 10.2       | RJ / NRJ               | rs699947, rs1570360 (AR) 2          | 10                   |
| Prakash            | 40  | 2015 | India   | Indian    | 37.1 ± 9.4        | AR / NRJ               | rs699947, rs1570360, rs3025039, rs144854329 (AR) 4 | 5                    |
| Prakash            | 41  | 2018 | India   | Indian    | 38.2 ± 11.6       | Graft failure / functioning graft | rs699947, rs1570360, rs3025039, rs144854329 (CR) 4 | 6                    |
| Gunesacar          | 42  | 2007 | Germany | Caucasian | 31.7 ± 0.7        | Graft failure / functioning graft | rs3025039 (AR) 1                | 6                    |
| Jimenez-Sousa      | 43  | 2012 | Spain   | Caucasian | 50.5 (16.6)*      | CRAD / non-CRAD         | rs699947 (CRAD-CR) 1             | 6                    |
| Lemos              | 23  | 2005 | Netherlands | Caucasian | 47.1 ± 13.5    | AR / Non-AR            | rs699947, rs1570360, rs25648 (AR) 3 | 7                    |
| Shahbazi           | 24  | 2002 | United Kingdom | Caucasian | 39.0 ± 15.3 | RJ / NRJ               | rs699947, rs1570360 (AR) 2          | 6                    |

VEGF: vascular endothelial growth factor; [R]: Reference; y: years; KT: kidney transplantation; RJ: rejection NRJ: non-rejection; AR: acute rejection; CR: chronic rejection; CRAD: chronic renal allograft dysfunction; n: number of studies; * median (range)
| First author | Ethnicity | AR/CR | VEGF SNPs      | Sample sizes | Statistical power | RJ  | NRJ | Total | Minor allele frequency | HWE P-value |
|--------------|----------|-------|----------------|--------------|-------------------|-----|-----|-------|-----------------------|-------------|
|              |          |       |                |              | (α = 0.05; OR 1.5) |     |     |       |                       |             |
|              |          |       |                |              | wt-wt             | wt-var | var-var | wt-wt | wt-var | var-var |       |                       |             |
|              |          |       |                |              |                   |       |       |       |                       |             |
| VEGF1 (rs699947+rs144854329) | 663      | 956   | 1,619          | 97.7†         |                   |       |       |       |                       |             |
| Jimenez-Sousa | Caucasian | CR    | rs699947       | 158 118 276   | 37.4              | 40  83 | 35  45 49 24 0.41 | 0.122 |
| Lemos        | Caucasian | AR    | rs699947       | 93 267 360    | 38.1              | 21  46 | 26  60 133 74 0.53 | 0.987 |
| Shahbazi     | Caucasian | AR    | rs699947       | 64 103 167    | 23.9              | 24  33 | 7   24 50 29 0.52 | 0.785 |
| Mittal       | Indian    | AR    | rs699947       | du du ----     | ----              | 10  23 | 11  30 71 55 0.58 | 0.412 |
| Prakash5     | Indian    | AR    | rs699947       | 76 196 272    | 31.4              | 23  31 | 22  38 119 39 0.50 | 0.0027 |
| Prakash5     | Indian    | AR    | rs144854329    | du du ----     | ----              | 19  34 | 23  39 101 56 0.59 | 0.591 |
| Prakash8     | Indian    | CR    | rs699947       | 98 174 272    | 35.1              | 13  52 | 33  48 98 28 0.44 | 0.288 |
| Prakash8     | Indian    | CR    | rs144854329    | du du ----     | ----              | 15  62 | 21  43 73 58 0.54 | 0.041 |
| Prakash8     | Indian    | AR    | rs699947       | du du ----     | ----              | 48  98 | 28  13 52 33 0.60 | 0.288 |
| Prakash8     | Indian    | AR    | rs144854329    | du du ----     | ----              | 58  73 | 43  21 62 15 0.47 | 0.008 |
| Prakash8     | Indian    | AR    | rs144854329    | 54 218 272    | ----              | 58  73 | 43  21 62 15 0.47 | 0.008 |
| Prakash8     | Indian    | CR    | rs1570360      | du du ----     | ----              | 47  38 | 8   118 119 30 0.34 | 0.999 |
| Prakash5     | Indian    | AR    | rs1570360      | du du ----     | ----              | 47  38 | 8   118 119 30 0.34 | 0.999 |
| Prakash5     | Indian    | CR    | rs1570360      | du du ----     | ----              | 48  98 | 28  13 52 33 0.60 | 0.288 |
| Prakash8     | Indian    | CR    | rs144854329    | du du ----     | ----              | 23  53 | 22  39 93 42 0.51 | 0.418 |
| Prakash5     | Indian    | AR    | rs3025039      | 265 290 555   | 65.2†             |       |       |       |                       |             |
| Gunesacar    | Caucasian | AR    | rs3025039      | 265 290 555   | 65.1              | 231 31 | 3   230 55 5 0.11 | 0.423 |
| Prakash5     | Indian    | AR    | rs3025039      | du ----        | ----              | 20  33 | 23  79 80 37 0.39 | 0.043 |
| Prakash8     | Indian    | CR    | rs3025039      | du ----        | ----              | 39  42 | 17  60 71 43 0.45 | 0.335 |

**Table 2.** Quantitative features of the included *VEGF* studies that examined associations with kidney transplantation outcome.

VEGF: vascular endothelial growth factor polymorphisms; AR: acute rejection; CR: chronic rejection; SNPs: single nucleotide polymorphisms; RJ: rejection; NRJ: non-rejection; HWE: Hardy-Weinberg Equilibrium; wt: wild-type; var: variant; du: duplicate; the 5 and 8 after Prakash indicate the last digit of publication year for these articles; values in bold indicate total sample sizes for each VEGF SNP group and significant departure from the HWE; † aggregate statistical power for the VEGF groups.
Sensitivity analysis was used to test for robustness of the summary effects. Publication bias was considered for significant (P < 0.05) comparisons with ≥ 10 studies. Significance was set at a two-sided P-value of < 0.05, except for heterogeneity estimation, which was set at Phet < 0.10. Data for the meta-analysis were analyzed using Review Manager 5.3 (Cochrane Collaboration, Oxford, England), SIGMASTAT 2.03, and SIGMAPLOT 11.0 (Systat Software, San Jose, CA).

Results

Search results and study features

Figure 1 outlines the study selection process in a flowchart following guidelines from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA; Reporting guidelines). Table S1 (Extended data) shows the initial search using combinations of four search strings applied to four databases resulted in 1,949 citations, followed by a series of omissions that mostly involved duplications (n = 1,924). The gray literature database (Mednar) yielded no additional papers for inclusion. Thus, the final number of included articles for this meta-analysis was seven.

Characteristics of the included studies

Of the seven articles, five examined more than one VEGF SNP (Table 1). The number of studies VEGF1 (rs699947 and rs144854329), VEGF2 (rs1570360) and VEGF3 (rs3025039) were 10, five and three, respectively (Table 2). Of the 10 VEGF1 studies, seven and three were in Indian and Caucasian populations, respectively. Of the five VEGF2 studies, three and two were in Indian and Caucasian populations, respectively. One Caucasian and two Indian studies comprised VEGF3. Table 1 shows two publications that investigated CR, which translated to three studies for VEGF1 (Table 2), otherwise, the rest focused on AR (Table 1 and Table 2).

Table 2 shows an aggregate total sample size (663 RJ/956 NRJ) and a statistical power of 97.7% for VEGF1. In contrast, both VEGF2 (105 RJ/254 NRJ) and VEGF3 (265 RJ/290 NRJ) were underpowered (40.5% and 65.2%). Mean age of the subjects was 39.96±6.6 years (± standard deviation) indicating a near to middle-age demographic profile of the KT subjects. The Clark-Baudouin scores (median 6.0, interquartile range 6.0–6.75) indicated that the methodological quality of the component

![Figure 1](image-url)
studies was moderate. Control frequencies deviated from the HWE in three studies (from two articles) for VEGF1, two studies for VEGF2, and one study for VEGF3.

Meta-analysis outcomes

VEGF1 associations with KT. Table S2 (Extended data) shows 32 comparisons, six of which were significant (P = 0.0009–0.04). Of the six, five were post-outlier derived and four survived the Bonferroni correction (Table 3). Of the four, three were in wt indicating increased risk (overall: 1.41, 95% CI 1.14-1.75, P = 0.002 [Figure 2]), Indian: OR 1.44, 95% CI 1.13-1.84, P = 0.004, CR: OR 2.10, 95% CI, P = 0.0009) and one in var, indicating reduced risk (Indian: OR 0.61, 95% CI 0.45-0.820, P = 0.001). Only the CR outcome had zero heterogeneity (I² = 0%).

VEGF2 associations with KT. Table S2 (Extended data) shows 18 comparisons, four of which were significant (P = 0.001–0.04), were in the wt model and had moderate heterogeneity (I² = 30%-51%). Three of the four were products of pre-outlier analysis, where the HW-compliant outcome (OR 1.39, 95% CI 1.01-1.91, P = 0.04) confirmed the overall outcome (OR 1.48, 95% CI 1.01-2.15, P = 0.04). The other overall outcome was post-outlier derived and survived the Bonferroni correction (OR 1.55, 95% CI 1.06-2.28, P = 0.02) contrasted with the non-significant Indian outcome (OR 1.36, 95% CI 0.72-2.58, P = 0.34).

VEGF3 associations with KT. Table S3 (Extended data) shows eight comparisons, two of which were significant (P = 0.008–0.01) but did not withstand Bonferroni correction. These two homogeneous (I² = 0%) pooled ORs indicated reduced risk in the dominant and codominant models (ORs 0.66-0.69, 95% CIs 0.47-0.92).

Summary of significant VEGF associations with KT. Table 3 summarizes the information on the 12 significant outcomes, five of which survived the Bonferroni correction, four in VEGF1 and one in VEGF2, all deemed robust. These outcomes identified three VEGF polymorphisms (rs699947, rs144854329 and rs1570360) that were associated with allograft rejection post-KT. VEGF1 subgroup outcomes identified CR associations and Indians to be at risk. Depending on the genetic model, the Indian population were both susceptible (wt: OR 1.44, 95% CI 1.13-1.84) and protected (var: OR 0.61, 95% CI 0.45-0.82).

Table 3. Summary of main outcomes of VEGF SNP associations with allograft rejection post-kidney transplantation (chronic + acute).

| SNP group | Genetic model | Comparison | Outlier status | n | OR  | 95% CI | P* | I² (%) | Analysis model | Sensitivity outcome |
|-----------|---------------|------------|----------------|---|-----|--------|----|--------|-----------------|-------------------|
| VEGF1     |               |            |                | 9 | 1.41| 1.14-1.75 | 0.002* | 0.17 | 31 | Fixed | Robust |
|           |               | Overall    | Post           | 2 | 2.10| 1.36-3.24 | 0.0009* | 0.50 | 0   | Fixed | Robust |
|           |               | Chronic rejection | Post | 5 | 0.61| 0.45-0.82 | 0.001* | 0.16 | 39 | Fixed | Robust |
|           |               | Indian     | Pre            | 7 | 1.44| 1.13-1.84 | 0.004* | 0.16 | 35 | Fixed | Robust |
|           |               | Overall    | Post           | 6 | 1.39| 1.07-1.81 | 0.02 | 0.23 | 28 | Fixed | Not robust |
| VEGF2     |               |            |                | 4 | 1.58| 1.19-2.09 | 0.001* | 0.12 | 49 | Fixed | Robust |
|           |               | Overall    | Post           | 5 | 1.48| 1.01-2.15 | 0.04 | 0.09 | 51 | Random | Not robust |
|           |               | Overall    | Pre            | 3 | 1.39| 1.01-1.91 | 0.04 | 0.24 | 30 | Fixed | Not robust |
|           |               | Caucasian  | Post           | 2 | 1.55| 1.06-2.28 | 0.02 | 0.19 | 42 | Fixed | Not robust |
| VEGF3     |               | Codominant | Overall        | 2 | 0.69| 0.53-0.91 | 0.01 | 0.36 | 0   | Fixed | Not robust |
|           |               | Dominant   | Overall        | 2 | 0.66| 0.47-0.92 | 0.01 | 0.33 | 0   | Fixed | Not robust |

VEGF: vascular endothelial growth factor gene; VEGF1: rs699947+rs144854329; VEGF2: rs1570360; VEGF3: rs3025039; wt: wild-type; var: variant; HW: Hardy-Weinberg; n: number of studies; OR: odds ratio; CI: confidence interval; P*: P-value for association; Phet: P-value for heterogeneity; I²: measure of variability; * values in bold survived the Bonferroni correction.
Table 1. Forest plot in the post-outlier overall outcome for the wt allele in VEGF1 (n = 9). Diamond denotes the pooled odds ratio (OR) indicating increased risk (1.41). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% confidence intervals (CI). The Z test for overall effect shows significance (P = 0.002). The χ²-square test outcome has low-level heterogeneity (P = 0.12). Subjecting these P-values to Bonferroni correction and sensitivity treatment raised the level of evidence of association. By design, such features are not present in the component single-study outcomes. This underpins the value of meta-analysis in systematically synthesizing primary study results and providing insight into associations of VEGF SNPs with allograft rejection post-KT.  Confounding outcomes between primary studies may be due to small sample sizes, hence, lack of power. Underpowered outcomes appear to be common in candidate gene studies and are prone to the risk of Type I error. In spite of the evidence for associations, the complexity of allograft rejection involves interactions between genetic and non-genetic factors allowing for the likelihood of environmental involvement. Gene-gene and gene-environment interactions have been reported to have roles in associations of other SNPs with post-KT allograft rejection. Two articles examined polymorphisms in other genes included interleukin 18 (IL18), transforming growth factor beta 1 (TGFBI), and angiotensin II receptor type 1 (AGTR1). None of the seven articles acknowledged gene-environment interactions. Four of the included articles mentioned haplotype analysis with three presenting haplotype data. Additional well-designed studies exploring other parameters would confirm or modify our results in this study and add to the extant knowledge about the association of the VEGF SNPs and renal allograft outcome.

**VEGF and renal allografts**

VEGF plays a crucial role in kidney physiology with its involvement in maintaining the integrity and permeability of the glomerular capillary basement membrane. Adaptive response of VEGF toward renal allograft tissue may be related to its angiogenic property on endothelial cells since VEGF contributes to tissue repair response of damaged capillaries. After KT, the recipient’s neutrophils and macrophages infiltrate the allograft after reperfusion of the transplanted tissue leading to the production of VEGF. Shahbazi et al. showed that genetically directed variations in VEGF production with increased frequency of VEGF producing alleles seemed to influence susceptibility to acute allograft rejection. However, Lemos et al. also suggested that renal allograft recipients with genetic potential for high VEGF production had significantly better graft survival compared to recipients with low VEGF production. Our results along the timeline of post-KT outcomes indicated increased risks, both for AR and CR in the wt allele, which agreed with Shahbazi et al. but contrasted with Lemos et al. However, the significance of our increased risk CR finding may require caution in its interpretation given the low number of studies (n = 2) and low statistical power (64.4%). More studies may be needed to clarify our CR outcome. In terms of ethnicity, Indians carriers of the wt CC genotype in rs699947 (−2578C/A), were afforded better graft survival than the CA and AA genotypes. In contrast, Shahbazi et al. found that the −2578 C allele (rs699947) and the −1154 G allele (rs1570360) were associated with increased risk of acute renal allograft rejection.

---

**Discussion**

**Summary of findings**

The five Bonferroni-filtered findings (wt and var alleles) were either products of outlier treatment and/or subgrouping. Subgrouping identified the ethnicity and rejection type that was significant, thus specifying associations of the VEGF polymorphisms with allograft rejection post-KT. Subgrouping provided contrasts regarding significant outcomes: (i) In VEGF1, significant in Indians (P = 0.001–0.004), non-significant in Caucasians (P = 0.78–1.00); (ii) in VEGF2, significant in Caucasians (P = 0.02) and non-significant in Indians (P = 0.34); (iii) in VEGF1, significant in CR (P = 0.0009), non-significant in AR (P = 0.12). Subjecting these P-values to Bonferroni correction and sensitivity treatment raised the level of evidence that facilitated interpretation with greater confidence. We have shown that meta-analytical tools such as subgrouping, outlier and sensitivity treatments are instrumental in generating evidence for association. By design, such features are not present in the component single-study outcomes. This underpins the value of meta-analysis in systematically synthesizing primary study results and providing insight into associations of VEGF SNPs with allograft rejection post-KT. Conflicting outcomes between primary studies may be due to small sample sizes, hence, lack of power. Underpowered outcomes appear to be common in candidate gene studies and are prone to the risk of Type I error. In spite of the evidence for associations, the complexity of allograft rejection involves interactions between genetic and non-genetic factors allowing for the likelihood of environmental involvement. Gene-gene and gene-environment interactions have been reported to have roles in associations of other SNPs with post-KT allograft rejection. Two articles examined polymorphisms in other genes that included interleukin 18 (IL18), transforming growth factor beta 1 (TGFBI), and angiotensin II receptor type 1 (AGTR1). None of the seven articles acknowledged gene-environment interactions. Four of the included articles mentioned haplotype analysis with three presenting haplotype data. Additional well-designed studies exploring other parameters would confirm or modify our results in this study and add to the extant knowledge about the association of the VEGF SNPs and renal allograft outcome.
in Caucasians conferring greater risk among wt homozygotes (-2578C/C and -1154 G/G) compared to -2578C/A and -1154G/ A heterozygous genotypes\textsuperscript{24}. These inconsistent associations among previous studies may be due to the variations in genetic background influenced by differential ethnicities of the patients.

**Strengths and limitations**

Interpreting our findings should consider its limitations and strengths. Strengths include: (i) VEGF1 combined sample sizes translated to high aggregate statistical power (97.7%); (ii) significant HWC outcomes validated the overall pooled effects in wt. These validations served to reduce the risk of genotyping errors and minimize methodological weaknesses in our study; (iii) subgroup outcomes in CR and Indians point to potential clinical utility in the genetics of renal transplantation; (iv) efficiency of outlier treatment was the key to generating associative significance and eliminating or reducing heterogeneity and (v) stability of the core overall outcomes are underpinned by surviving the Bonferroni correction (minimizing Type 1 error risk) and robustness (determined with sensitivity treatment).

On the other hand, limitations include: (i) all the component studies were underpowered; (ii) most of the moderately significant outcomes (67%) were non-robust.

**Conclusions**

To our knowledge, this is the first meta-analysis to examine associations between VEGF SNPs and risk of allograft rejection post-KT. Risks for renal allograft rejection associated with VEGF\textsuperscript{1} polymorphisms were shown to be increased up to 1.6-fold for the wt allele and 39% reduced for the var allele. Subgroups found to be susceptible were the Indian population and CR. These highly significant and robust core effects could render the VEGF\textsuperscript{1} polymorphisms useful as a prognostic biomarker in allograft rejection post-KT.

**Data availability**

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

**Extended data**

Dryad: Influence of polymorphisms in the vascular endothelial growth factor gene on allograft rejection after kidney transplantation: a meta-analysis, https://doi.org/10.5061/dryad.gqnk98skz\textsuperscript{25}.

This project contains the following extended data:

- S1 Table Overall, modified and subgroup outcomes for VEGF1.
- S2 Table Overall, modified and subgroup outcomes for VEGF2.
- S3 Table Overall, modified and subgroup outcomes for VEGF3.

**Reporting guidelines**

Dryad: PRISMA checklist for ‘Influence of polymorphisms in the vascular endothelial growth factor gene on allograft rejection after kidney transplantation: a meta-analysis’, https://doi.org/10.5061/dryad.gqnk98skz\textsuperscript{26}.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

**Acknowledgements**

We’d like to thank A Kunjantarachot for her invaluable contributions at the incipient stages of the draft preparation.

---

**References**

1. Levey AS, Atkins R, Coresh J, et al.: Chronic kidney disease as a global public health problem: approaches and initiatives - a position statement from Kidney Disease Improving Global Outcomes. Kidney Int. 2007; 72(3): 247-259. PubMed Abstract | Publisher Full Text

2. Howard K, Salkeld G, White S, et al.: The cost-effectiveness of increasing kidney transplantation and home-based dialysis. Nephrol (Carlton). 2009; 14(1): 123-132. PubMed Abstract | Publisher Full Text

3. Critchley WR, Fildes JE: Graft rejection - endogenous or allogeneic? Immunology. 2012; 136(1): 123-132. PubMed Abstract | Publisher Full Text | Free Full Text

4. Goldberg Rj, Weng Fl, Kandula P: Acute and Chronic Allograft Dysfunction in Kidney Transplant Recipients. Med Clin North Am. 2016; 100(3): 487-503. PubMed Abstract | Publisher Full Text

5. Bhownik DM, Dinda AK, Mahanta P, et al.: The evolution of the Banff classification schema for diagnosing renal allograft rejection and its implications for clinicians. Indian J Nephrol. 2010; 20(1): 2-8. PubMed Abstract | Publisher Full Text | Free Full Text

6. Seeger H, Lindenmeyer MT, Cohen CD, et al.: Lymphotoxin expression in human and murine renal allografts. PLoS One. 2018; 13(1): e0189396. PubMed Abstract | Publisher Full Text | Free Full Text

7. Bestard O, Cruzado JM, la Franquesa M, et al.: Biomarkers in renal transplantation. Curr Opin Organ Transplant. 2010; 15(4): 467-473. PubMed Abstract | Publisher Full Text

8. Dmitrienko S, Hoar DJ, Balshaw R, et al.: Immune response gene polymorphisms in renal transplant recipients. Transplantation. 2005; 80(12): 1773-1782. PubMed Abstract | Publisher Full Text

9. Pasculus M, Theruvath T, Kawai T, et al.: Strategies to improve long-term outcomes after renal transplantation. N Engl J Med. 2002; 346(8): 580-590. PubMed Abstract | Publisher Full Text

10. Krüger B, Schröppel B, Murphy BT: Genetic polymorphisms and the fate of the transplanted organ. Transplant Rev (Orlando). 2008; 22(2): 131-140. PubMed Abstract | Publisher Full Text

11. Perco P, Oberbauer R: Integrative analysis of -omics data and histologic scoring in renal disease and transplantation: renal histogenomics. Semin Nephrol. 2010; 30(5): 520-530. PubMed Abstract | Publisher Full Text | Free Full Text

12. Wilson AG, Symons JA, McDowell TL, et al.: Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A. 1997; 94(7): 3195-3199. PubMed Abstract | Publisher Full Text | Free Full Text

13. Herath S, Erlich J, Au AYM, et al.: Advances in Detection of Kidney Transplant Injury. Mol Diagn Ther. 2016; 20(3): 333-351. PubMed Abstract | Publisher Full Text

14. Ferrara N: Molecular and biological properties of vascular endothelial growth factor. J Mol Med (Berl). 1999; 77(7): 527-543. PubMed Abstract | Publisher Full Text
Open Peer Review

Current Peer Review Status:  

Version 1

Reviewer Report 08 March 2022

https://doi.org/10.5256/f1000research.30745.r119145

© 2022 Karimzadeh I. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Iman Karimzadeh

Department of Clinical Pharmacy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

The authors in this meta-analysis focused on the possible association between vascular endothelial growth factor gene polymorphism and allograft rejection after kidney transplantation. They demonstrated that risks for renal allograft rejection associated with VEGF polymorphisms were shown to be increased up to 1.6-fold for the wild type allele and 39% reduced for the variant allele. The topic is novel and interesting. The meta analysis was done properly. The manuscript is well-written. Therefore, the manuscript can be considered for indexing after implementing all comments and revisions.

○ The first reference related to the frequency of acute rejection in kidney transplantation should be added into the first paragraph of the introduction section.

○ The second reference related to the role of urine VEGF level in allograft rejection should be added and briefly discussed in the second paragraph of the introduction section.

References
1. Mottaghi S, Sagheb MM, Azarpira N, Abdizadeh F, et al.: Association between the Three Polymorphisms of the Glucocorticoid Receptor Gene and the Early Clinical Outcome in Kidney Transplantation Patients. *Iran J Med Sci*. 46 (6): 444-453 PubMed Abstract | Publisher Full Text
2. Peng W, Chen J, Jiang Y, Shou Z, et al.: Acute renal allograft rejection is associated with increased levels of vascular endothelial growth factor in the urine. *Nephrology (Carlton)*. 2008; 13 (1): 73-9 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?  
Yes

Is the study design appropriate and is the work technically sound?  
Yes
Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Pharmacogenetics in the setting of kidney transplantation.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

---

The article written by Puntilla and colleagues is very interesting and challenging. The subject is very actual, searching for new biomarkers in attempt to maximize long survival after kidney transplantation. The approach of many research teams is to reveal new biomarkers with impact on kidney allograft rejection. The VEGF gene polymorphisms completes other discussed biomarkers with impact on acute and chronic kidney rejection, like microRNAs, anti-angiotensin II type 2 receptor antibody and urinary cytokines, MIG, IP-10 etc.

Transplant genetics is complex. We have to have in mind genetic background of chronic renal disease and also the genetic status of transplantation per se.

The meta analysis of VEGF SNPs is comprehensive well-documented using updated and accurate statistical methods. The literature search is well represented and well documented. The conclusions are clear. The information is valuable but reading the article could be difficult for a person who is not used with such extensive statistical analyses. I strongly recommend the
indexing of this article with only one comment: it is very captivating but difficult to follow in its current format and must be converted to an easier content.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology, Immunogenetics, Transplantation, Molecular Biology, Virology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

The benefits of publishing with F1000Research:

• Your article is published within days, with no editorial bias
• You can publish traditional articles, null/negative results, case reports, data notes and more
• The peer review process is transparent and collaborative
• Your article is indexed in PubMed after passing peer review
• Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com