BMC Cardiovascular Disorders

RESEARCH

Open Access

VEGF-A cis-located SNPs on human chromosome 6 associated with VEGF-A plasma levels and survival in a coronary disease cohort

J. C. Meza-Alvarado[1], A. P. Pilbrow[2], C. M. Frampton[2], V. A. Cameron[2], A. M. Richards[2], R. W. Troughton[2], R. N. Doughty[3], R. A. Page[1], B. Mallard[1], C. Bromhead[1] and B. R. Palmer[1*]

Abstract

Background Cardiovascular disease (CVD) is the leading cause of death worldwide. Risk stratification of CVD patients may be improved by predictive biomarkers, including genetic markers. Elevated circulating vascular endothelial growth factor A (VEGF-A) levels have been linked to CVD development. We explored whether single nucleotide polymorphisms (SNPs) at the *VEGFA* locus on human chromosome 6 were associated with VEGF-A levels and clinical outcomes in established CVD. VEGF-A levels were compared between coronary heart disease patients and heart healthy controls.

Methods Imputed genotypes of 30 SNPs from the *VEGFA* region for 1935 patients from the Coronary Disease Cohort Study (CDCS) and 1183 individuals from the Canterbury Healthy Volunteers Study (HVOL) were analysed for associations with cardiometabolic parameters. Association with clinical endpoints was assessed using Kaplan-Meier analysis and multivariate regression models. To validate the findings from imputed data, DNA samples of 2027 CDCS patients and 227 HVOL participants were manually genotyped for variants rs6921438 and rs7767396. Baseline plasma VEGF-A assayed by ELISA in 227 HVOL participants was compared with levels in 549 CDCS patients.

Results Manual genotyping showed rs6921438 AA and rs7767396 GG genotype groups had lower VEGF-A levels at baseline (**CDCS**: rs6921438 AA (27.7 pg/mL), AG (43.3 pg/mL), GG (63.2 pg/mL), $p = 4.49 \times 10^{-22}$; rs7767396: GG (27.4 pg/mL), AG (42.8 pg/mL), AA (61.5 pg/mL) $p = 3.47 \times 10^{-21}$; **HVOL** rs6921438 AA (12.8 pg/mL), GA (19.9 pg/mL), GG (26.4 pg/mL) $p = 0.021$; rs7767396 GG (12.6 pg/mL), AG (19.6 pg/mL), AA (25.9 pg/mL) $p = 0.029$). In the CDCS cohort rs6921438 AA was associated with increased risk of all-cause death ($p = 0.03$); non ST-elevated myocardial infarction (NSTEMI, $p = 0.0003$), heart failure (HF, $p = 0.035$) and major adverse cardiovascular events ($p = 0.032$); rs7767396 GG was associated with increased NSTEMI ($p = 0.001$) and HF ($p = 0.023$) risk; rs6921438 AA (Hazard Ratio (HR) = 6.55 $p = 0.017$), rs7767396 GG (HR = 0.149, $p = 0.017$) and VEGF-A (HR = 2.55, $p = 0.018$) were independent HF admission risk predictors.

*Correspondence:
B. R. Palmer
b.palmer@massey.ac.nz

Full list of author information is available at the end of the article

Conclusions Variants rs6921438 and rs7767396 are associated with plasma VEGF-A levels. Both SNPs and VEGF-A may be useful in prognosis for HF after acute coronary events.

Keywords Vascular endothelial growth factor, Single nucleotide polymorphism, Outcome, Prognosis, rs6921438, rs7767396

Introduction

Cardiovascular disease (CVD) is an important contributor to health deficits in New Zealand and worldwide [1]. Prediction of outcomes after CVD events may be aided by biomarkers [2]. Members of the vascular endothelial growth factor (VEGF) family have previously been proposed for risk stratification in CVD [3–5]. VEGF-A is a key factor in blood vessel formation (angiogenesis) and collateral circulation (arteriogenesis), mediated by binding to the receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR) [6]. These receptors are found on the surface of endothelial and non-endothelial cells [7]. VEGFR1 participates in angiogenesis, while VEGFR2 is the primary inducer of VEGF-mediated blood vessel growth [8–10]. Additionally, VEGFR1 interacts with the co-receptor neuropilin-1, which selectively potentiates VEGFR2-mediated vascular permeability, and endothelial cell motility in vascular development [11, 12]. Furthermore, VEGFR1 has a soluble splice variant (sFlt-1) that acts as a decoy receptor, decreasing VEGF-A plasma concentration and limiting VEGFR2 binding [7, 8, 13].

Increased plasma and tissue levels of VEGF-A have been observed in coronary heart disease (CHD), stroke, and heart failure (HF) [14–17]. Additionally, elevated VEGF-A levels have been associated with various CVD risk factors including smoking, hypercholesterolaemia, diabetes, hypertension, metabolic syndrome, and hyperglycaemia [6, 13, 14]. Increased VEGF-A activity impacts the vascular system by increasing inflammatory molecule activity (e.g. TNF-α, IL-6) leading to increased vascular dilation, adhesion protein expression and trans-endothelial lipid migration which promote atherosclerotic lesion development [6, 14, 18–22].

The *VEGFA* gene has a 16.3 kb coding region located at 6p21.1 on the short arm of chromosome 6, including eight exons and seven introns [23, 24]. Factors that upregulate *VEGFA* gene expression include the hypoxia inducible factor, p53 polymorphisms, thyroid stimulating hormone, oestrogen levels and low oxygen tension [8, 22, 23, 25]. VEGF-A circulating levels are influenced by several SNPs. Four common variants (rs6921438, rs4416670, rs6993770 and rs10738760) distributed across three chromosomes have been independently associated with circulating VEGF-A levels and explain up to 48% of the heritability of serum VEGF-A levels [26]. A meta-analysis of genome wide association study (GWAS) data suggested ten SNPs contributed up to 52% of variance in total circulating VEGF-A [27].

Individual studies have also identified that SNPs at the *VEGFA* gene locus are associated with CVD [24–26] and CVD risk factors such as lipid metabolites [26–28] and coronary disease biomarkers [24–29]. Furthermore, elevated levels of VEGF-A may contribute to CVD onset or progression [5, 28, 29]. We explored the relationships of 30 *VEGFA* variants with cardiometabolic variables, including plasma concentrations of VEGF-A, in post-acute coronary syndrome (ACS) patients and heart healthy matched controls [3, 4, 30–32]. SNPs were also analysed for associations with clinical endpoints in the post-ACS cohort.

Materials and methods

Study population

Coronary disease cohort study (CDCS)

The CDCS recruited 2140 patients with a diagnosis of ACS [ST elevation myocardial infarction (STEMI), non-STEMI (NSTEMI), and unstable angina, (UA)] were enrolled in the Coronary Disease Cohort Study (CDCS) at Christchurch Hospital or Auckland City Hospital (New Zealand), between July 2002 and January 2009. Inclusion criteria at index admission included ischaemic discomfort plus one or more of electrocardiogram (ECG) change (ST-segment depression or elevation of ≥ 0.5 mm, T-wave inversion of ≥ 3 mm in ≥ 3 leads, or left bundle branch block), elevated levels of cardiac markers, a history of coronary disease, age ≥ 65 years, and a history of diabetes or vascular disease [32]. Patients with serious co-morbidity (e.g. end-stage renal failure, cancer) that limited their life expectancy to < 3 years, were excluded. Recruitment included a wide spectrum of age, both genders and significant sub-groups with established risk factors for CHD including hypertension and type II diabetes. Demographic and clinical data were collected at baseline, including blood pressure, height, weight, ECG, echocardiography, family and personal medical history and medication regimes. Plasma samples were assayed for natriuretic peptides (ANP, NT-ANP, BNP, NTproBNP, CNP, NT-CNP) and other cardiometabolic analytes (total cholesterol, creatinine, urate, troponin I, aldosterone, endothelin and adrenomedullin) [31, 32]. Patients were followed for a median of 5.04 (0.08–9.49) years. Patients attended follow-up clinics at 3–5 months and 12–14 months post-onset of ACS. Plasma levels of VEGF-A were assayed as described previously [4]. Ethnicity was self-declared and categorised as European, Māori/Pasifika (Pacific Islander), Asian

and Middle Eastern/Latin American/African (MELAA). Standardised transthoracic echocardiography was performed at baseline and at each follow-up clinic either at Christchurch Hospital or University of Auckland clinics, as described previously [31, 32]. The study was approved by the New Zealand (NZ) Multi-Region Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12605000431628 on 16 September 2005). All participating patients provided written, informed consent. The investigation conforms to the principles outlined in the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46.

Canterbury healthy volunteers study (HVOL)

Participants ($n = 250$) matched by age and gender to CDCS patients were selected from the HVOL Cohort ($n = 3{,}358$) [33]. Participants in the HVOL were randomly selected from the electoral rolls of Canterbury, New Zealand. Individuals from the electoral roll were assigned a unique identifier and chosen at random using the random selection module in an SPSS database. Those selected were approached by mail and invited to participate in a screening questionnaire, thus excluding those with prior hospital admissions and diagnoses for CVD. CVD risk factors, anthropometric measures, personal health information and family history of cardiovascular events were recorded for each participant. Plasma sample aliquots were taken at a baseline clinic and biobanked at -80 °C and assayed for different analytes as previously described [33]. Recruitment commenced in January 2002, and clinical events were documented from the NZ Health Information Service (NZHIS) over a median follow-up of 9.2 years (range 20 days – 16.5 years). The study was approved by the New Zealand Health and Disability Ethics Committee (Reference CTY/01/05/062) and registered with the Australian New Zealand Clinical Trials Registry (ACTRN1260500448640). All participants gave written, informed consent. The investigation conforms to the principles outlined in the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46.

Clinical events

Clinical events in CDCS were documented at scheduled follow-up clinic visits, through consulting patient notes and Hospital Patient Management System (PMS) databases plus regular updates and corroboration acquired from the NZHIS. Endpoints of interest included death from any cause, myocardial infarction (MI, classified as ST elevated [STEMI], non-ST elevated [NSTEMI] or unspecified), stroke (ischaemic or haemorrhagic), HF and unstable angina. STEMI patients were defined by the presence of typical cardiac ischemic symptoms, ischemic change in two or more contiguous EKG leads, and peak elevation of plasma creatine kinase.>400 U/L as reported elsewhere [32, 34]. UA was defined according to published guidelines based on the duration and severity of angina as graded by each of the 2 enrolment teams according to the Canadian Cardiovascular Society classification [35, 36]. HF was recorded for the purpose of assessing associations with clinical outcomes and was obtained by scrutinising data from (i) outpatient attendance or phone calls with subjects, family member or primary care physician; (ii) Hospital PMS; (iii) NZHIS records (case note examination) and (iv) standardised questionnaires mailed to participants at year 2 and year 3 post index discharge [35]. The composite variable major adverse cardiovascular event (MACE) included patients presenting any of the following: any cause of death, any type of MI (STEMI or NSTEMI) or any type of stroke (ischaemic or hemorrhaegic). Survival times were calculated from the date of index admission. The investigation conforms to the principles outlined in the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46.

Genotype imputation

Variants selected for imputation were 30 previously identified SNPs mapping to the *VEGFA* locus, with reported associations with cardiometabolic variables or CVD risk [3, 4, 37–46], or overlapping genomic regulatory regions (e.g. transcription factor binding motifs, enhancer signatures) based on genome assembly GRCh38/hg38 from the University of California, Santa Cruz Genome Browser (http://genome.ucsc.edu) and Ensembl databases [47, 48]. Imputation was carried out across human chromosome 6 using the Michigan Imputation Server, which implemented the 'minimac4' algorithm [49]. Strand-aligned genotype data were loaded into the server. The imputation was performed using the HRC r1.1 2016 reference panel and GRCh37/hg19 array build in 'Quality Control and Imputation' mode (population = other/mixed). Phasing was performed with Eagle v2.4. All biallelic variants with an imputation quality threshold of INFO score ≥ 0.3 were extracted. Genotype data was obtained for the 30 SNPs for 1935 patient DNA samples from the CDCS cohort and 1138 individuals from the HVOL cohort.

SNP genotyping

To validate imputed genotype data, manual genotyping (TaqMan assays, ThermoFisher Scientific; Applied Biosystems; Foster City, NJ, USA) of two survival-associated SNPs (C__11542106_10 [rs6921438] and C__29965406_20 [rs7767396]) was performed for 2027 DNA samples from the CDCS cohort and 227 samples from the HVOL cohort. Reactions were performed on a Roche LightCycler LC96 system (Roche Diagnostics Ltd., Rotkreuz, Switzerland) using a 10 µL reaction volume in 96 well plates using TaqMan Master Mix (Applied

Biosystems) SNP-specific primers and probes and 1 ng of genomic DNA. As quality control, a random selection of 10% of samples were re-genotyped with 99.5% concordance with the original genotypes.

Plasma VEGF-A assay

Plasma samples were collected and stored at -80˚C as previously described [3]. Levels of VEGF-A at baseline from 549 CDCS participants have been reported previously [4]. A subset of 223 plasma samples from the HVOL cohort were assayed for comparison. Briefly, after thawing, each plasma aliquot was vortexed for 30 s followed by centrifugation at 5000 rpm for 15 s at 4 °C to sediment cellular debris. The HVOL plasma samples then underwent assay for VEGF-A by a quantitative sandwich ELISA using the manufacturer's instructions (R&D Systems Europe, Abingdon, UK). VEGF-A concentration is reported as pg/mL (detectable range 0.09–115 pg/mL).

Statistical analysis

Univariate analyses were performed to test associations between genotypes of all 30 imputed SNPs and baseline data for anthropomorphic measurements, analyte levels and echocardiographic measurements using $\chi 2$ and ANOVA tests, with age as a covariate and Bonferroni correction. Skewed data were log-transformed before analysis and geometric means with 95% confidence intervals reported. The survival of groups was compared using Kaplan-Meier analysis and the log-rank test.

SNP genotypes were analysed using an additive model unless indicated otherwise. Candidate CVD risk variants were selected from among the imputed SNPs if they had significant association ($p < 0.05$) or trended towards significance ($p < 0.1$) with cardiac risk markers or with plasma levels of VEGF-A. Five SNP variants were selected for further analysis based on association with plasma concentrations of VEGF-A and natriuretic peptides (Additional File 1 Supplementary Table 1). These 5 variants were analysed for univariate association with survival using the Kaplan-Meier log-rank test and by multivariate Cox proportional hazards models to identify independent associations between genotype groups and all-cause mortality. The multivariate survival analyses included the established predictors: age, gender, previous MI, beta-adrenergic blocker treatment, physical activity, and plasma NTproBNP levels. Two variants presenting significant univariate and independent survival associations in a multivariate model (Fig. 2; Table 4) were selected for manual genotyping. The ANOVA, Kaplan-Meier and Cox proportional hazards multivariate analyses were repeated using the manual genotypes. In these analyses 6 clinical outcome endpoints were considered (all-cause mortality, STEMI, NSTEMI, unstable angina, MACE and HF). All analyses were performed using SPSS version 28.0.1.1 (IBM, Armonk, USA). Statistical significance was set at the 5% level ($p < 0.05$).

Table 1 Baseline characteristics of the CDCS cohort

Variables	*n*	Mean ± SD or *n* (%)
Anthropometric		
Male gender	2026	1453 (71.7%)
Age	2026	66.7 ± 12.2
Ethnicity (European, Māori & Pasifika, Asian, MELAA)	2026	90%, 5.9%, 3.8%, 0.3%
Previous MI	2012	597 (29.5%)
Previous Heart failure	2016	188 (9.3%)
Antecedent Hypertension	2009	1048 (51.7%)
Body mass index (kg/m^2)	1998	27.5 ± 4.99
Tobacco (smoker, ex-smoker, never smoked)	2026	6.3%, 57.4%, 36.3%
Alcohol (drinker, ex-drinker, non-drinker)	2023	63%, 12%, 25%
Cardiovascular Readmission after discharge	2026	1335 (65.9%)
All-cause death	2026	472 (23.3%)
Medications		
Antithrombotic	2026	1951 (96.3%)
Angiotensin converting enzyme inhibitors	2022	1153 (57%)
Beta blocker	2022	1740 (86.1%)
Mineralocorticoid receptor antagonists	2022	18 (0.89%)
Diuretic	2022	547 (27.1%)
Statin	2022	1789 (88.5%)
Other antihypertensive drugs	2022	557 (27.5%)

Abbreviations: MELAA: Middle Eastern Latin American African, MI: myocardial infarction, SD: Standard deviation

Genomic context analysis

The genomic contexts for rs6921438 and rs7767396 were explored to identify if the variants overlapped transcription factor binding motifs or regulatory regions involved with CVD risk. This assessment involved using the human genome assembly GRCh38/hg38 from the University of California, Santa Cruz Genome Browser (http://genome.ucsc.edu), the associated JASPAR database of transcription factor motifs, the Ensembl database and HaploReg databases [48, 50, 51].

Results

Baseline characteristics of study cohorts

Baseline characteristics of the CDCS cohort are given in Table 1. The population is predominantly of European ethnicity (90%) and male (71.7%). During the follow-up period (median of 5.04 years, range of 0.08–9.49 years) the clinical outcomes with highest incidence from the CDCS cohort ($n = 2026$) were MACE ($n = 862$ or 40.3%) followed by NSTEMI ($n = 488$ or 24.1%). all-cause death ($n = 472$ or 23.3%) and heart failure ($n = 392$ or 8.59%). Additionally, discharge medications most prescribed were antithrombotic therapy (96.3%) followed by lipid

lowering medication (88.5%) and antihypertensive agents (57%).

Baseline characteristics of the HVOL subset are described in Table 2. The population was mostly European (98.4%) and male (64%). There were no significant differences for age and gender between the CDCS patients and HVOL cohort subset (Age $p = 0.112$, Gender $p = 0.08$). As expected, compared to the CDCS participants, HVOL participants had comparatively lower incidence of high total cholesterol (31%, $p = 4.16 \times 10^{-12}$), hypertension (30.5%, $p = 1.24 \times 10^{-10}$) and diabetes (5.2%, $p = 2.42 \times 10^{-7}$). Additionally, low levels of medication usage were observed with antihypertensives (27.6%, $p = 7.54 \times 10^{-8}$) being the most common.

SNP genotype frequencies

Following initial ANOVA tests on the imputed genotype data obtained on 1935 CDCS patients for 30 SNPs, five variants (rs4513773, rs6921438, rs7763440, rs7767396, rs11757868) were associated with ten variables including plasma levels of VEGF-A and natriuretic peptides at baseline (Additional File 1 Supplementary Table 1). Imputed genotype variants rs6921438 and rs7767396 showed significant associations in univariate and multivariate survival analyses with all-cause mortality (Fig. 2; Table 2). Imputed genotype data for 1183 HVOL individuals showed five variants (rs4513773, rs6921438, rs7763440, rs7767396, rs11757868) were associated with ANP levels (data not shown). To confirm the imputed genotype findings, manual genotyping was performed for rs6921438 and rs7767396 on DNA samples from 2026 CDCS patients and 227 HVOL individuals. These variants were selected due to their reported involvement with CVD risk pathways [40, 52–55] and their association with mortality.

Table 2 Baseline characteristics of the HVOL subset

Variables	*n*	Mean ± SD or *n* (%)
Anthropometric		
Male gender	250	160 (64%)
Age	250	68.9 ± 8.25
Ethnicity (European, Māori & Pasifika, Indian)	250	98.4%, 1.6%, 0%
BMI (kg/m^2)	250	26.4 ± 4.13
MI Family history	248	105 (42%)
Tobacco (smoker, ex-smoker, never smoked)	249	5%, 35.6%, 59.4%
Alcohol (drinker, ex-drinker, non-drinker)	250	78.8%, 4.4%, 16.8%
Previous disease history		
Hypertension	249	76 (30.5%)
High Cholesterol	245	76 (31%)
Diabetes	250	13 (5.2%)
Medication		
Antithrombotic	250	31 (12.4%)
Lipid lowering	250	45 (18%)
Antihypertensive	250	69 (27.6%)

Abbreviations: BMI: body mass index, MI: myocardial infarction, SD: Standard Deviation

The CDCS manual and imputed genotype frequencies for rs6921438 and rs7767396 are summarised in Additional File 2 Supplementary Table 2. For the CDCS and HVOL cohorts, manual and imputed genotype frequencies for rs6921438 and rs7767396 were concordant (Additional File 2 Supplementary Table 2). The manual genotyped frequencies for both SNPs in both cohorts have similar frequencies as those observed in the European group from the 1000 Genomes Project data [48].

rs6921438 and rs7767396 metabolite associations

A summary of the ANOVA analyses using imputed genotype data for rs4513773, rs6921438, rs7763440, rs7767396 and rs11757868 in the CDCS cohort is shown in Additional File 1 Supplementary Table 1. Manual genotyping showed mean VEGF-A levels trended to progressively decrease with the addition of minor alleles for each SNP (Table 3, rs6921438 $p = 4.49 \times 10^{-22}$, rs7767396 $p = 3.47 \times 10^{-21}$). Specifically, CDCS patients with the rs6921438 AA genotype had lower plasma VEGF-A levels than those with the GA (Fig. 1A, $p = 7.36 \times 10^{-11}$) and GG (Fig. 1A, $p = 1.49 \times 10^{-22}$) groups. Similarly, CDCS patients with the rs7767396 GG genotype had lower VEGF-A levels compared to the AG (Fig. 1B, $p = 1.98 \times 10^{-10}$) and AA (Fig. 1B. $p = 6 \times 10^{-6}$) groups. Additionally, rs6921438 GG and rs7767396 AA were associated with lower systolic blood pressure (Table 3).

CDCS VEGF-A levels were compared with VEGF-A levels measured in the HVOL subset (Fig. 1). As expected from a heart healthy cohort, HVOL individuals had lower VEGF-A levels compared to the CDCS cohort (Fig. 1). When comparing the three genotype groups for each SNP in the HVOL subset, mean VEGF-A levels progressively decreased with the addition of minor alleles for each SNP (rs6921438, $p = 0.021$, rs7767396 $p = 0.029$). HVOL individuals with the rs6921438 AA genotype (VEGF-A – 12.8 pg/mL ± 1.61) had the lowest levels while the GA (19.9 pg/mL ± 1.83) and GG groups (26.4 pg/mL ± 5.63) had higher levels. Likewise, individuals carrying the rs7767396 GG genotype (VEGF-A – 12.6 pg/mL ± 1.74) had lower VEGF-A levels than the AG (19.6 pg/mL ± 1.81) and AA (25.9 pg/mL ± 5.53) groups. For the HVOL imputed genotype data, only ANP levels were significantly associated with five SNPs (rs4513773, rs6921438, rs7763440, rs7767396 and rs11757868). No variable besides VEGF-A was associated with manual genotyping data on rs6921438 and rs776736 for the HVOL subset.

Table 3 CDCS baseline characteristics, drug treatment and neurohormonal data stratified by manual genotype group for (a) rs7767396 and (b) rs6921438

a) rs7767396 Genotypes							
Variable	***N***	**AA**	*n*	**AG**	***n***	**GG**	***p***
Anthropometric Variables							
Age (years)	577	65.7 ± 12.1	985	66.9 ± 12.2	465	67.2 ± 12.3	0.073
Activity	575	19.7%, 11%, 15%, 54.3%	983	21.6%, 13.6%, 13.3%, 51.5%	465	23.7%, 12.7%, 14.4%, 49.2%	0.443
Male Gender	577	435 (75.4%)	985	691 (70.2%)	465	328 (70.5%)	0.069
Ethnicity (European, Māori/Pasifika, Asian, MELAA)	577	87.5%, 5.7%, 6.5%, 0.3%	985	90.4%, 6.1%, 3%, 0.5%	465	92%, 5.6%, 2.4%, 0%	**0.008**
Height (m)	571	1.71 ± 0.092	972	1.69 ± 0.097	460	1.70 ± 0.098	0.092
Weight (kg)	573	80.5 ± 16.1	973	79.3 ± 17.0	459	79.7 ± 17.3	0.428
BMI (kg/m^2)	571	27.6 ± 5.04	969	27.5 ± 5.02	459	27.4 ± 4.88	0.818
Systolic BP (mmHg)	570	126.9 ± 20.7	963	129.7 ± 22.1	453	129.7 ± 22.1	**0.031**
Diastolic BP (mmHg)	570	74.8 ± 11.5	963	74.9 ± 12.1	453	74.3 ± 11.5	0.696
Beta blocker use	577	497 (86.1%)	985	835 (84.8%)	465	411 (88.4%)	0.179
Statin use	577	515 (89.3%)	985	871 (88.4%)	465	408 (87.7%)	0.744
Previous MI	570	175 (30.7%)	982	275 (28%)	461	147 (31.9%)	0.261
Tobacco use (Current, Ex Smoker, Non-smoker)	577	6.9%, 57.7%, 35.4%	985	6%, 57.5%, 36.5%	465	6.2%, 56.8%, 37%	0.941
Analytes							
Cholesterol (mmol/L)	454	4.94 ± 1.19	774	4.89 ± 1.17	364	4.84 ± 1.18	0.462
Creatinine (mmol/L)	559	99.8 ± 48.6	958	97.9 ± 49.3	451	101.6 ± 56.0	0.434
Urate (mmol/L)	330	0.369 ± 0.101	583	0.372 ± 0.100	270	0.377 ± 0.097	0.646
Troponin I (ng/L) [$]	549	35.5 (23.5–47.4)	944	49.7 (20.6–78.9)	441	32.5 (20.2–44.8)	0.517
ANP (pg/mL) [$]	575	41.7 (39.2–44.1)	980	43.6 (41.6–45.6)	460	45.1 (42.3–47.9)	0.210
NT-ANP (pmol/L) [$]	575	1.29 (1.21–1.38)	979	1.33 (1.25–1.39)	460	1.43 (1.31–1.55)	0.205
BNP (pmol/L) [$]	575	24.1 (21.7–26.5)	980	26.7 (24.5–28.8)	460	28.5 (25.04–31.9)	0.157
NTproBNP (pg/ml) [$]	575	119.3 (108–130.7)	980	136.9 (124.9–149.1)	460	149.4 (129.3–169.4)	0.111
CNP (pmol/L) [$]	568	0.641 (0.605–0.678)	964	0.668 (0.639–0.697)	453	0.677 (0.629–0.725)	0.505
NT-CNP (pmol/L) [$]	567	23.4 (21.5–25.2)	962	22.5 (20.9–24.2)	453	23.3 (21.2–25.3)	0.223
Aldosterone (pmol/L) [$]	562	170.2 (145.2–195.3)	956	165.4 (158.4–172.5)	445	161.1 (150.9–171.2)	0.598
Endothelin(pmol/L) [$]	575	2.66 (2.58–2.74)	980	2.66 (2.59–2.73)	460	2.74 (2.65–2.83)	0.252
Adrenomedullin (pg/ml) [$]	558	8.56 (8.09–9.03)	942	8.48 (8.19–8.77)	450	8.68 (8.18–9.19)	0.693
VEGF-A (pg/mL) [$]	161	61.5 (53.9–69.1)	253	42.8 (39.6–46.1)	119	27.4 (24.9–29.9)	$\mathbf{3.47 \times 10^{-21}}$
b) rs6921438 genotypes							
Variable	**n**	**GG**	**n**	**GA**	**n**	**AA**	**p**
Anthropometric Variables							
Age (years)	529	65.7 ± 12.1	983	67.1 ± 12.1	514	66.8 ± 12.5	0.090
Activity (Sed, Mild, Mod, Act)	527	20.3%, 10.2%, 15.4%, 54.1%	981	21.1%, 13.5%, 13.3%, 52.1%	514	23.5%, 13.8%, 14.4%, 48.3%	0.253
Male Gender	529	400 (75.6%)	983	689 (70.1%)	514	364 (70.8%)	0.066
Ethnicity (European, Māori & Pasifika, Asian, MELAA)	529	89.4%, 4%, 6.2%, 0.4%	983	90.2%, 6.6%, 2.8%, 0.4%	514	90.3%, 6.4%, 3.1%, 0.2%	**0.013**
Height (m)	523	1.71 ± 0.091	972	1.69 ± 0.096	507	1.70 ± 0.099	0.064
Weight (kg)	525	80.5 ± 16.1	973	79.2 ± 16.8	506	80.0 ± 17.7	0.320
BMI (kg/m^2)	523	27.6 ± 4.91	969	27.5 ± 4.97	506	27.6 ± 5.13	0.865
Systolic BP (mmHg)	522	126.4 ± 20.5	962	129.7 ± 21.9	501	129.9 ± 22.5	**0.010**
Diastolic BP (mmHg)	522	74.4 ± 11.5	962	75.1 ± 11.9	501	74.4 ± 11.8	0.465
Beta blocker use	529	455 (86%)	983	840 (85.5%)	514	449 (87.4%)	0.600
Statin use	529	472 (89.2%)	983	866 (88.1%)	514	454 (88.3%)	0.803
Previous MI	522	158 (30.3%)	980	287 (29.3%)	510	152 (29.8%)	0.922
Tobacco use (Current, Ex Smoker, Non-smoker)	529	7.4%, 57.6%, 35%	983	5.9%, 57.6%, 36.5%	514	6%, 56.8%, 37.2%	0.793
Analytes							
Cholesterol (mmol/L)	419	4.96 ± 1.19	767	4.87 ± 1.17	404	4.86 ± 1.19	0.363
Creatinine (mmol/L)	514	98.7 ± 45.4	956	97.9 ± 48.9	497	102.8 ± 58.8	0.214

Table 3 (continued)

Urate (mmol/L)	304	0.372 ± 0.102	585	0.37 ± 0.100	295	0.377 ± 0.097	0.610
Troponin I (ng/L) [$]	504	31.5 (22.3–40.7)	943	52.4 (22.8–81.9)	486	31.9 (20.7–43.2)	0.786
ANP (pg/mL) [$]	527	41.4 (38.8–43.9)	979	43.9 (41.9–45.9)	508	44.6 (41.9–47.2)	0.269
NT-ANP (pmol/L) [$]	527	1.28 (1.19–1.37)	978	1.34 (1.27–1.41)	508	1.41 (1.3–1.52)	0.293
BNP (pmol/L) [$]	527	23.5 (21.01–26.04)	979	26.8 (24.7–29.02)	508	28.4 (25.2–31.6)	0.074
NTproBNP (pg/ml) [$]	527	117.1 (105.3–128.8)	979	138 (125.9–150.1)	508	147.7 (129.1–166.2)	0.087
CNP (pmol/L) [$]	520	0.639 (0.600–0.678)	965	0.670 (0.641–0.699)	499	0.676 (0.631–0.720)	0.535
NT-CNP (pmol/L) [$]	519	22.7 (21.4–23.9)	963	22.5 (20.9–24.2)	499	24.03 (21.5–26.5)	0.246
Aldosterone (pmol/L) [$]	515	168.9 (141.7–196.1)	955	166.8 (159.7–173.9)	492	160.9 (151.3–170.5)	0.207
Endothelin (pmol/L) [$]	527	2.66 (2.58–2.74)	979	2.65 (2.59–2.72)	508	2.74 (2.66–2.83)	0.153
Adrenomedullin (pg/ml) [$]	510	8.68 (8.17–9.19)	944	8.48 (8.19–8.77)	495	8.56 (8.09–9.03)	0.970
VEGF-A (pg/mL) [$]	148	63.2 (55.1–71.3)	257	43.3 (40.1–46.6)	130	27.7 (25.3–30.2)	**4.49×10^{-22}**

$Log10 transformed p-values are reported

Mean ± standard deviation or Mean (95% CI range) or incidence (%) are reported

Significantly associated variables and their p-values are shown in **bold**

Abbreviations: Act: active (≥ 30 min on ≥ 3 days/week), ANP: atrial natriuretic peptide, BP: blood pressure, BMI: body mass index, BNP: B-type natriuretic peptide, CNP: C-type natriuretic peptide, MELAA: Middle Eastern/Latin American/African, MI: Myocardial infarction, Mod: moderate (≥ 30 min on 2 days/week), NT-ANP Amino terminal atrial natriuretic peptide, NT-CNP: Amino terminal C-type natriuretic peptide NTproBNP = amino-terminal pro-B type natriuretic peptide, Sed: Sedentary, VEGF-A: Vascular endothelial growth factor A

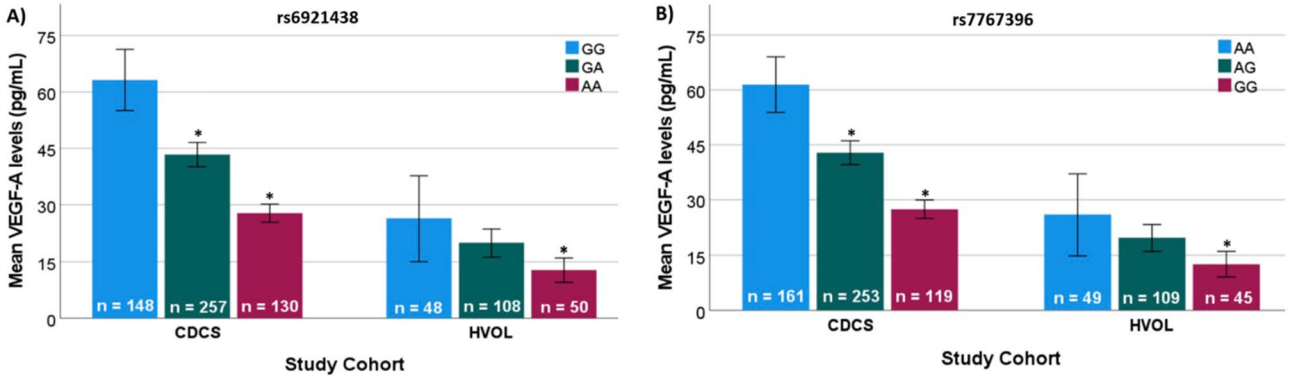

Fig. 1 VEGF-A levels of CDCS and HVOL cohorts for manual genotypes of (**A**) rs6921438 and (**B**) rs7767396. Bars represent geometric means with 95% CI error bars. *Indicates genotype groups compared to the reference genotype group (blue bar) of the respective cohort ($p < 0.05$)

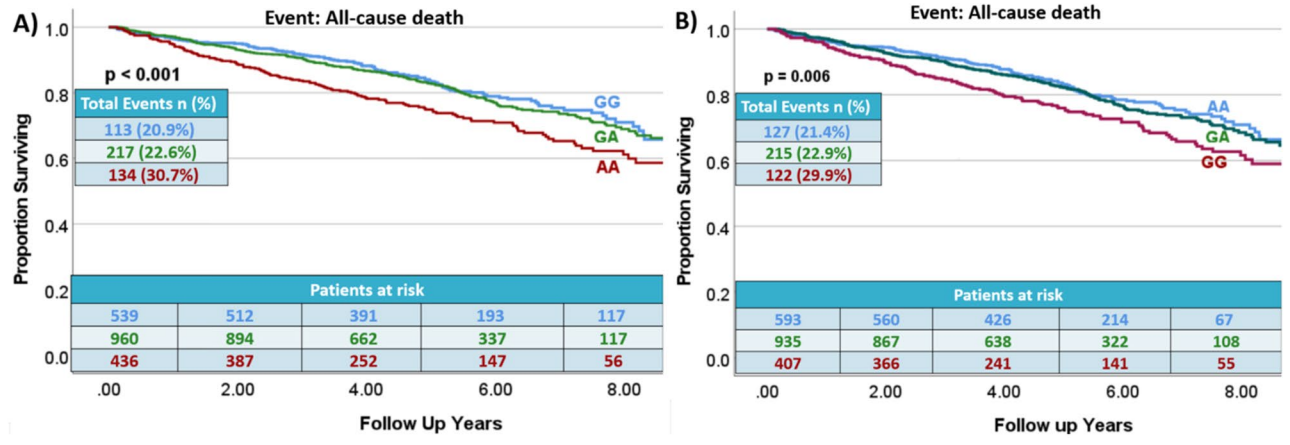

Fig. 2 All-cause death survival plot for CDCS cohort stratified by imputed genotypes of **A**) rs6921438 and **B**) rs7767396. Genotypes are colour coded blue for homozygous reference, green for heterozygous and red for homozygous for the minor allele. Patients are risk reported for every 2-year interval

Univariate survival association of selected SNPs in the CDCS cohort

Survival analyses showed imputed rs6921438 and rs7767396 genotypes (Fig. 2) were associated with risk of all-cause death, with those homozygous for the minor allele (rs6921438 AA and rs7767396 GG) more likely to die. Consistent with this, Kaplan-Meier survival analyses using manual genotyping data revealed patients carrying rs6921438 AA were more likely to die during the follow up period (Fig. 3A), had higher likelihood of having a readmission for NSTEMI (Fig. 3B), HF (Fig. 3C) or MACE (Fig. 3D). Patients with the rs7767396 GG genotype were more often readmitted for NSTEMI (Fig. 4A) or HF (Fig. 4B), but this genotype was not significantly associated with risk of all-cause death or MACE.

Multivariate survival association of selected SNPs in the CDCS cohort

Cox proportional hazards models for all-cause mortality showed imputed rs6921438 and rs7767396 genotypes were associated with increased risk (Table 4). When using manual genotype data in a similar model, neither SNP was significantly associated with increased risk of mortality (Table 5). We observed that baseline plasma VEGF-A was an independent predictor of risk of all-cause mortality (Table 5, HR = 2.69, $p = 0.002$). In a model for heart failure readmission (Table 6), rs6921438 AA (HR = 6.55 $p = 0.017$) and elevated VEGF-A levels (HR = 2.59, $p = 0.018$) were associated with increased risk, while rs7767396 GG associated with reduced readmission risk (HR = 0.149, $p = 0.017$).

Genomic context for rs6921438 and rs7767396

Both rs6921438 and rs7767396 are located downstream from *VEGFA* (Fig. 5). The SNPs are approximately 1.4 kb from each other between long noncoding RNAs *LINC0512* and *C6orf23* (Fig. 5), approximately 170 kb from the *VEGFA* locus. Two motifs that overlap with rs6921438 are binding sites for transcription factors FOXF2 and FOXH3. FOXF2 has the conserved function of participating in mural cell development and, according to the HaploReg database, the A allele of rs6921438 has increased FOXF2 binding activity compared to the G allele [50, 56]. Loss of FOXF2 function is associated

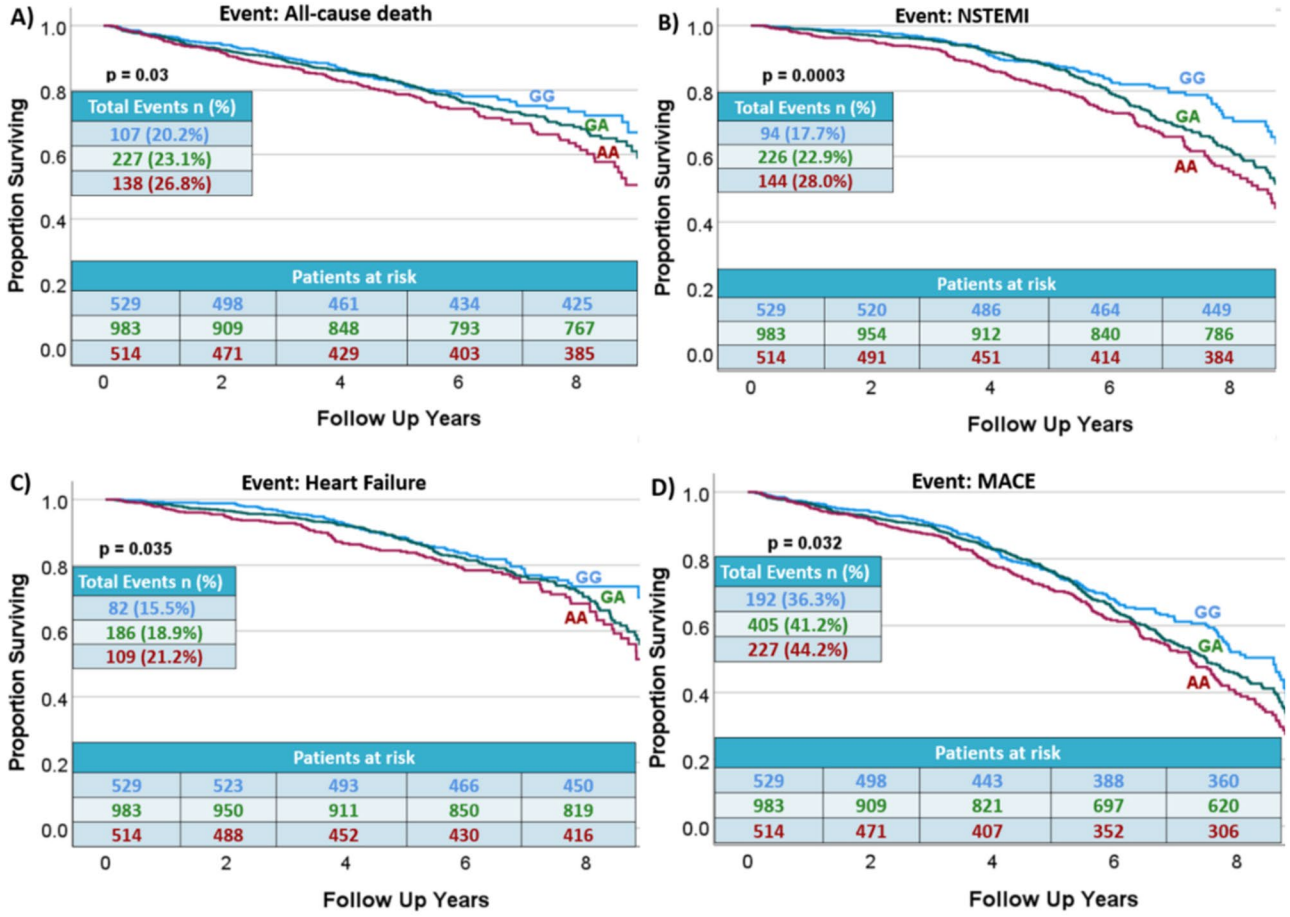

Fig. 3 Survival plots of CDCS rs6921438 manual genotypes for **A**) all-cause death, **B**) NSTEMI **C**) heart failure and **D**) MACE. Genotypes are colour coded blue for GG, green for GA and red for AA

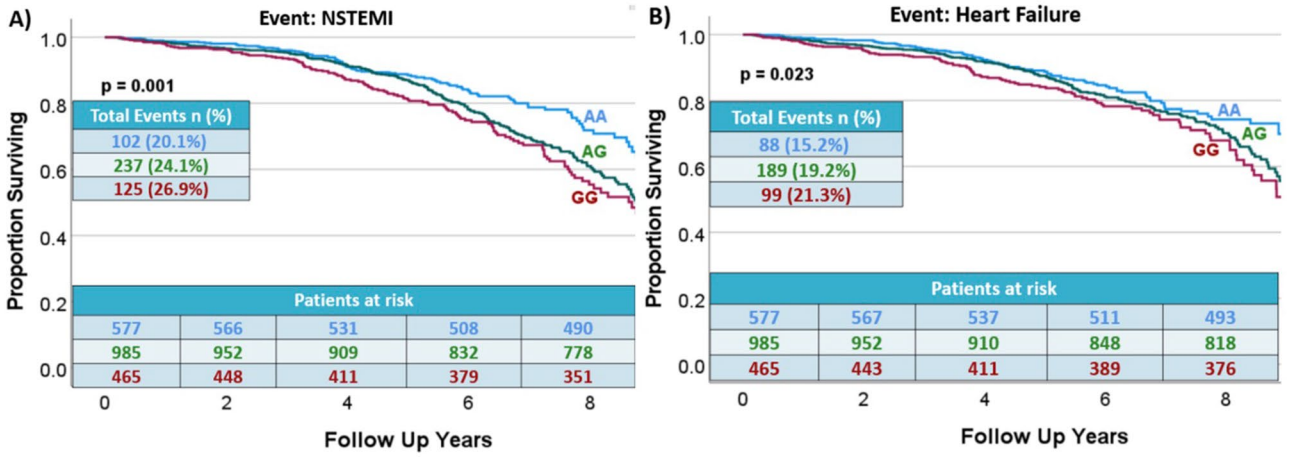

Fig. 4 Survival plots of CDCS rs7767396 manual genotypes for **A**) NSTEMI and **B**) heart failure. Genotypes are colour coded blue for AA, green for AG and red for GG

Table 4 Multivariate regression model for all-cause mortality in the CDCS cohort using manual genotypes for rs6921438 and rs7767396 ($n = 506$, 163 (32.2%) events)

Predictor	Coeff.	SE	Wald	*P*-value	HR	95% CI for HR	
						Lower	Upper
Gender	0.078	0.188	0.171	0.679	1.081	0.748	1.56
Ethnicity			0.988	0.804			
European v Māori/Pasifika	-0.525	1.07	0.241	0.623	0.592	0.073	4.803
European v Asian	0.880	1.03	0.732	0.392	2.41	0.321	18.1
European v MELAA	-6.32	207.8	0.001	0.976	0.002	2.56×10^{-180}	1.27×10^{174}
Physical Activity (scale 1–4)[$$]	-0.325	0.069	22.5	**2.09×10^{-6}**	0.722	0.631	0.826
Previous MI	0.527	0.167	9.95	**0.002**	1.69	1.22	2.35
Age	0.062	0.012	28.6	**8.79×10^{-8}**	1.06	1.04	1.09
Body mass index	-0.056	0.023	6.28	**0.012**	0.945	0.904	0.988
Urate	1.17	0.835	1.95	0.162	3.21	0.625	16.5
Creatinine	0.004	0.001	8.62	**0.003**	1.004	1.001	1.007
Beta blocker	-0.065	0.237	0.076	0.783	0.937	0.589	1.49
Statin	-0.263	0.202	1.69	0.194	0.769	0.517	1.14
Log10 NTproBNP[$]	0.809	0.255	10.01	**0.002**	2.24	1.36	3.69
Log10 VEGF-A[$]	0.993	0.325	9.34	**0.002**	2.69	1.43	5.102
rs6921438 genotype			3.33	0.189			
GG v GA	0.026	0.507	0.003	0.959	1.02	0.380	2.77
GG v AA	0.862	0.646	1.78	0.182	2.36	0.668	8.39
rs7767396 genotype			2.4	0.301			
AA v AG	0.057	0.493	0.013	0.908	1.06	0.403	2.78
AA v GG	-0.671	0.643	1.09	0.297	0.511	0.145	1.804
Time to sampling	0.004	0.008	0.251	0.617	1.004	0.989	1.02

$Hazard Ratio represents the change in risk for every 10-fold increase in NTproBNP or VEGF-A level

$$Score of 1 = sedentary, 2 = < 30 min activity on > 2 days/week, 3 = ≥ 30 min on 2 days/week, 4 = ≥ 30 min on ≥ 3 days/week

Significant variables and their p-values are in **bold**

Abbreviations: CI = confidence interval, Coeff = Coefficient, HR = Hazard Ratio, MI = Myocardial Infarction, NTproBNP = amino-terminal pro-B type natriuretic peptide, SE = standard error, VEGF-A = Vascular endothelial growth factor A

with CVD pathogenesis, however an increase in FOXF2 binding may result in increased inflammatory signaling [56, 57]. This suggests that increased activity of FOXF2 could influence downstream regulation of inflammatory genes (IL-6, TNF-α), which are well established drivers of VEGF-A production [22, 58, 59]. Additionally, Azimi-Nezhad et al. (2013) identified that the A allele of rs6921438 could interact with a chromosome 8 SNP (rs6993770) and a chromosome 6 SNP (rs4416670, ~24.8 kb downstream of rs6921438) to increase IL-6

Table 5 Multivariate regression model for all-cause mortality in the CDCS cohort using imputed genotypes for rs6921438 and rs7767396 ($n = 509$, 166 (32.6%) events)

Predictor	Coeff.	SE	Wald	*P*-value	HR	95% CI for HR	
						Lower	Upper
Gender	0.015	0.190	0.007	0.935	1.02	0.700	1.47
Ethnicity			0.923	0.820			
European v Māori/Pasifika	-0.797	1.08	0.602	0.438	0.451	0.060	3.38
European v Asian	-7.23	226.3	0.001	0.975	0.001	1.85×10^{-196}	2.85×10^{189}
European v MELAA	-1.39	1.484	0.888	0.346	0.247	0.013	4.53
Physical Activity (scale 1–4)[$$]	-0.309	0.069	20.1	**7.16×10^{-6}**	0.734	0.642	0.840
Previous MI	0.590	0.167	12.5	**4.06×10^{-4}**	1.803	1.301	2.501
Age	0.064	0.012	30.6	**3.08×10^{-8}**	1.07	1.042	1.09
Body mass index	-0.053	0.021	6.09	**0.014**	0.948	0.909	0.989
Urate	1.52	0.840	3.27	0.071	4.57	0.881	23.7
Creatinine	0.005	0.001	11.9	**0.001**	1.005	1.002	1.008
Beta blocker	-0.128	0.230	0.310	0.578	0.880	0.561	1.38
Statin	-0.195	0.199	0.962	0.327	0.823	0.557	1.22
Log10 NTproBNP[$]	0.860	0.253	11.5	**0.001**	2.36	1.44	3.88
Log10 VEGF-A[$]	0.974	0.295	10.9	**0.001**	2.65	1.49	4.73
rs6921438 genotype			7.34	**0.026**			
GG v GA	-0.199	0.477	0.175	0.676	0.819	0.322	2.09
GG v AA	-1.47	0.649	5.13	**0.024**	0.230	0.064	0.820
rs7767396 genotype			10.1	**0.006**			
AA v AG	0.074	0.488	0.023	0.879	1.08	0.414	2.804
AA v GG	1.56	0.646	5.82	**0.016**	4.76	1.34	16.9
Time to sampling	0.003	0.008	0.202	0.653	1.003	0.988	1.02

$Hazard Ratio represents the change in risk for every 10-fold increase in NTproBNP or VEGF-A level

$$Score of 1 = sedentary, 2 = < 30 min activity on > 2 days/week, 3 = ≥ 30 min on 2 days/week, 4 = ≥ 30 min on ≥ 3 days/week

Significant variables and their p-values are in **bold**

Abbreviations: CI = confidence interval, Coeff = Coefficient, HR = Hazard Ratio, MI = Myocardial Infarction, NTproBNP = amino-terminal pro-B type natriuretic peptide, SE = standard error, VEGF-A = Vascular endothelial growth factor A

levels and TNF-α levels [60]. Overall, rs6921438 A's interaction with SNPs or transcription factor binding could be linked to CVD risk and VEGF-A production mechanisms as illustrated in Supplementary Fig. 1.

Comparatively, rs7767396 does not overlap with an epigenetic region, but it can affect a binding motif for STAT3, a cardiac transcription factor [61]. Additionally, the G allele can affect the binding of NF-AT1 which is linked to lower VEGF-A levels [50, 62]. NF-AT1 is a transcription factor involved in signaling that induces proinflammatory cytokine expression [63]. STAT3 upregulates the hypoxia responsive factor 1 alpha (HIF-1α), a well-established inducer of VEGF-A production [61]. In theory, reduced binding to STAT3 and NF-AT1 caused by rs7767396 G could contribute to the reduction of hypoxia and inflammation which could affect downstream VEGF-A production. These potential links are illustrated in Supplementary Fig. 1.

Discussion

Lower plasma VEGF-A levels were univariately associated with rs6921438 AA and rs7767396 GG in samples from patients in the CDCS cohort. In univariate survival analyses both SNPs were associated with clinical outcomes. rs6921438 AA was associated with increased risk of all-cause mortality, NSTEMI, HF and MACE readmission risk (Fig. 3). rs7767396 GG was associated with increased NSTEMI and HF readmission risk (Fig. 4). In multivariate analysis higher NTproBNP and VEGF-A levels, but not rs6921438 or rs7767396, were independent markers for increased risk of all-cause mortality and HF readmission (Tables 5 and 6).

Upon comparing VEGF-A levels by genotype, we observed a trend of mean VEGF-A levels progressively decreasing with the addition of minor alleles for rs6921438 and rs7767396 in both the CDCS and HVOL cohorts. GWAS analyses using healthy individuals have observed that rs6921438 A is associated with lower serum VEGF-A levels [26, 27] and this variant can explain 41.2% of VEGF-A serum level variance [26]. A similar trend on the reduction of plasma VEGF-A levels was noted for rs7767396's G allele in two oncological cohorts [62]. The impacts of specific alleles have been noted for other VEGF-A related SNPs on chromosome 6 (rs2010963, rs4416670, rs1740073, rs699947) whose major alleles have been associated with increased VEGF

Table 6 Multivariate regression model for heart failure readmissions in the CDCS cohort including manual genotypes for rs6921438 and rs7767396 ($n = 462$, 109 (23.6%) events)

Predictor	Coeff.	SE	Wald	*P*-value	HR	95% CI for HR	
						Lower	Upper
Gender	0.277	0.241	1.32	0.250	1.32	0.823	2.114
Ethnicity			6.41	0.093			
European v Māori & Pasifika	1.09	0.683	2.58	0.108	2.99	0.785	11.434
European v Asian	2.18	1.09	3.97	0.046	8.91	1.03	76.645
European v MELAA	-4.71	204.8	0.001	0.982	0.009	3.9×10^{-177}	1.9×10^{172}
Physical Activity (scale 1–4)[$$]	-0.397	0.086	21.3	$\mathbf{3.95\times10^{-6}}$	0.672	0.568	0.796
Previous MI	0.673	0.203	10.9	**0.001**	1.96	1.31	2.918
Age	0.063	0.014	19.3	$\mathbf{1.07\times10^{-5}}$	1.065	1.03	1.095
Body mass index	-0.012	0.030	0.153	0.695	0.988	0.931	1.049
Urate	2.97	1.09	7.51	**0.006**	19.6	2.33	165.220
Creatinine	0.002	0.002	1.23	0.267	1.002	0.998	1.006
Beta blocker	-0.589	0.279	4.46	**0.035**	0.555	0.322	0.958
Statin	0.101	0.263	0.147	0.702	1.106	0.661	1.851
Log10 NTproBNP[$]	1.75	0.358	23.7	$\mathbf{1.1\times10^{-6}}$	5.72	2.83	11.547
Log10 VEGF-A[$]	0.952	0.401	5.63	**0.018**	2.59	1.18	5.680
rs6921438 genotype			7.29	**0.026**			
GG v GA	0.607	0.680	0.797	0.372	1.83	0.484	6.959
GG v AA	1.88	0.790	5.66	**0.017**	6.55	1.39	30.792
rs7767396 genotype			7.72	**0.021**			
AA v AG	-0.455	0.655	0.482	0.488	0.635	0.176	2.292
AA v GG	-1.902	0.800	5.65	**0.017**	0.149	0.031	0.716
Time to sampling	0.021	0.010	4.59	**0.032**	1.02	1.002	1.041
LVEF at baseline	0.001	0.008	0.006	0.937	1.001	0.985	1.016

$Hazard Ratio represents the change in risk for every 10-fold increase in NTproBNP or VEGF-A level

$$Score of 1 = sedentary, 2 = < 30 min activity on > 2 days/week, 3 = ≥ 30 min on 2 days/week, 4 = ≥ 30 min on ≥ 3 days/week

Significant variables and their p-values are in **bold**

Abbreviations: CI = confidence interval, Coeff = Coefficient, LVEF = left ventricular ejection fraction, MI = Myocardial Infarction, NTproBNP = amino-terminal pro-B type natriuretic peptide, SE = standard error, VEGF-A = Vascular endothelial growth factor A

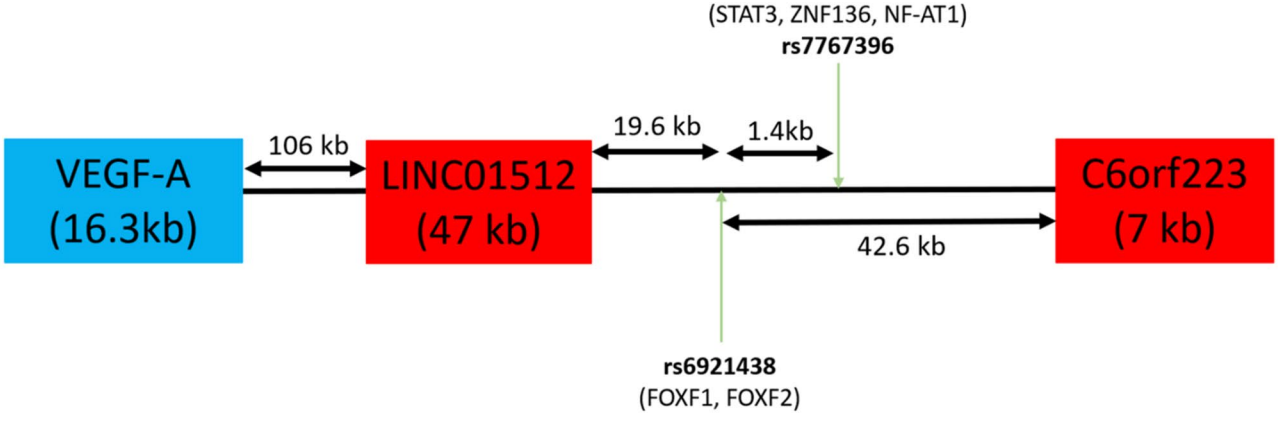

Fig. 5 Schematic representation of *VEGFA* region of human chromosome 6 (not to scale). SNPs analysed in the present study are shown in bold. Red boxes indicate lncRNAs. Blue box - coding gene for VEGF-A. Green arrows point to individual SNP locations. Transcription Factor binding sites overlapping analysed SNPs are named

serum levels [26, 27, 38, 64]. Our results support lower VEGFA plasma levels are associated with the minor alleles of rs6921438 and rs7767396.

The current work used imputed genotype data to select risk SNP candidates and explore further associations, prior to manual genotyping. We observed an association between rs6921438 AA and rs7767396 GG imputed genotype data and risk of all-cause mortality (Fig. 2; Table 4). Univariate survival analysis using manual genotyping data confirmed rs6921438 and rs7767396 genotypes were

associated with 4 outcomes (death, NSTEMI, HF, MACE) or 2 outcomes (HF, MACE), respectively (Figs. 3 and 4). A study of CAD patients identified that within a 5 year follow-up period rs2010963 CC/GC genotype, located within exon 1 of the *VEGFA* gene, was associated with increased risk of CAD-related death [42]. The same study showed the minor C allele to be a high-risk allele for cardiac death. Other studies report the minor allele genotypes of *VEGFA* locus SNPs have been associated with increased stroke risk (rs699947 and rs3025039) [37] and CHD (rs699947 and rs1570360) [43, 65]. Furthermore, assessment of multiple readmission risks in our study suggests rs6921438 and rs7767396 genotypes may have utility in risk stratification. Our results agree with studies on the influence of minor alleles of VEGF-A related SNPs on CVD related clinical outcomes.

The manual genotypes of rs6921438 and rs7767396, and established CVD risk predictors and VEGF-A levels were included in multivariate regression hazards models for multiple clinical endpoints. While neither variant was an independent predictor for risk of all-cause death, we observed higher VEGF-A and NTproBNP were associated with increased mortality. In a HF multivariate model, VEGF-A and NTproBNP also behaved as independent predictors. These findings agree with current knowledge on NTproBNP's value as a cardiac biomarker [66]. rs6921438 AA was associated with increased HF readmission risk, while rs7767396 GG was a predictor for reduced HF readmission risk. A previous study identified a *VEGFA* promoter SNP (rs699947) as an independent predictor of mortality in male non-diabetic participants in the CDCS cohort [4]. The use of imputed genotyping data proved useful to identify variants likely to be relevant to disease outcome that were then further explored following the determination of manual genotypes. Multivariate results suggest rs6921438 and rs7767396 may not be potential risk stratification markers for death.

Our multivariate model with HF as the endpoint showed the homozygous minor allele genotypes were associated with different HF risk effect direction for each SNP (rs6921438 AA increased HF risk, rs7767396 GG reduced HF risk). However, elevated VEGF-A levels were associated with increased HF risk. In this scenario, the SNPs independent behaviour may be influenced by each minor allele interacting with VEGF-A, other metabolites, or different CVD risk molecular pathways. For instance, the rs6921438 A allele has been associated with increased inflammatory cytokine (TNF-α and IL-6) levels in a healthy population [60]. Inflammation is key in increasing VEGF-A expression and activity [22, 58, 67]. Additionally, lower plasma VEGF-A levels have been linked with the rs6921438 A allele, which is also associated with decreased HDL and increased LDL levels in healthy European ancestry cohorts [40]. Notably, VEGF-A can reduce lipoprotein lipase activity, resulting in higher circulating LDL levels that confer CVD risk [18, 28]. Univariate findings correlate rs6921438 AA with lower baseline VEGF-A levels, but in the lead up to an HF event the AA group appears to confer increased HF risk, while being linked to higher VEGF-A. It is possible that the changes of direction of the effect of rs6921438 AA genotype that we observed between survival analyses may be attributed to HF onset features (cardiac stress, hypoxia, and inflammation). These factors may have a more pronounced effect over VEGF-A prior to a clinical event, which could alter rs6921438 AA's influence over baseline VEGF-A. This suggests that over time rs6921438 AA may contribute to the pathophysiological microenvironment associated with HF onset.

For its part, rs7767396 AA has been shown to be associated with higher VEGF-A levels than the AG and GG genotypes in cancer patients [62]. Additionally, the rs7767396 G allele is associated with reduced NF-AT1 binding. NF-AT1 is a transcription factor involved in heart development, heart failure and inflammatory pathways [62, 63]. There are no reports of this variant's involvement in CVD onset. rs7767396 is located in a binding motif for STAT3 (Fig. 5), a transcription factor that upregulates hypoxia responsive factor 1 alpha (HIF-1α), a well-established inducer of VEGF-A expression [61]. Our univariate data agrees that rs7767396 AA is associated with higher VEGF-A levels. When progressing towards HF onset rs7767396 GG was independently associated with higher VEGF-A levels, while also conferring reduced risk of disease. It is plausible that the reduced HF risk observed in our multivariate model is due to reduced NF-AT1 binding [62]. There are mechanisms by which rs6921438 and rs7767396 influence VEGF-A levels that differ according to the phenotype of a specific clinical outcome. It is possible that the minor alleles behave in one way at baseline, while in CVD onset, they may interact with known VEGF-A inducers (e.g. hypoxia, cytokines) to increase VEGF-A levels, thus contributing to the interaction between hypoxia, inflammation, and lipid metabolism pathways. Overall, rs7767396 G and rs6921438 A appear to be risk alleles of interest when HF onset biomarkers go above a certain threshold.

This study benefitted from access to imputed genotype data which allowed an efficient approach towards selecting SNPs for further analysis. The well characterised patient cohorts were robustly selected and intensively followed up. However, several limitations were identified. There are missing data for some analytes in the CDCS and HVOL cohorts which limit the power of multivariate models. Despite this, all models included non-VEGF-A analytes that affect CVD clinical outcomes (e.g. beta blocker treatment, creatinine, urate). Both cohorts studied are dominated by European participants which limits

the extrapolation of results to other populations. Furthermore, cohorts focusing on HF or subclinical atherosclerosis would aid in clarifying the impact of VEGF-A and VEGF-A eQTL SNP's on other CVDs. Lastly, blood samples from the CDCS cohort were collected at varying times after the index event, adjustment for time to sampling was included in statistical analysis to mitigate any confounding that might arise due to this.

Conclusion

In summary we report an association between rs6921438 and rs7767396 genotypes and VEGF-A plasma levels in post-ACS patients and age- and gender-matched heart healthy controls. Our findings also show the homozygous minor allele genotypes of both SNPs and VEGF-A levels are associated with the risk of heart failure following ACS, and therefore may have potential as markers for prognostic risk stratification. Further studies in other cohorts may provide validation of these findings in other populations.

Abbreviations

ACS	Acute coronary syndrome
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
BMI	body mass index
BNP	B-type natriuretic peptide
BP	Blood pressure
CDCS	Coronary Disease Cohort Study
CHD	Coronary heart disease
CI	Confidence Interval
CNP	C-type natriuretic peptide
Coeff	Coefficient
CVD	Cardiovascular disease
ECG	Electrocardiogram
GWAS	Genome wide association study
HF	Heart failure
HR	Hazard Ratio
HVOL	Canterbury Healthy Volunteers Study
IL-6	Interleukin 6
MACE	Major adverse cardiovascular events
MELAA	Middle Eastern/Latin American/African
MI	Myocardial infarction
NSTEMI	Non ST-elevated myocardial infarction
NT-ANP	Amino terminal atrial natriuretic peptide
NT-CNP	Amino terminal C-type natriuretic peptide
NTproBNP	Amino-terminal pro-B type natriuretic peptide
SD	Standard deviation
SE	Standard error
SNP	Single nucleotide polymorphism
TNF-α	Tumour necrosis factor alpha
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor A
VEGFR-1	Vascular endothelial growth factor receptor 1
VEGFR-2	Vascular endothelial growth factor receptor 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12872-025-04751-3.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

The authors thank participants in the studies, Christchurch Heart Institute Translational Biodiscovery unit staff for hormone and biochemical assays, study coordinators of the Christchurch Heart Institute and Auckland Cardiovascular Research Group for assistance with the recruitment and follow-up of the patients, Christchurch Heart Institute Omics laboratory staff for support in DNA and plasma sample access. The authors acknowledge Dr Vinicius Tragante (deCODE genetics, Iceland) for genotype imputation of SNP variants.

Author contributions

B.R.P and J.C.M.A. wrote the original draft manuscript text Conceptualization: B.R.P, A.M.R., V.A.C, J.C.M.A., A.P. P., R.A.P., C.B., B.L.M.Data curation: B.R.P., C. M. F., J.P., A. P. P.Formal analysis: B.R.P., C. M. F., A. P. P., J.C.M.A. Funding acquisition: B.R.P., J.C.M.A., R.N.D., A.M.R., V.A.CInvestigation: B.R.P., J.C.M.A., A. P. P.Methodology: A. P. P., R.N.D., A.M.R., V.A.C, R.W.T., Project administration: B.R.P., J.C.M.A., A. P. P., A.M.R., Supervision: B.R.P., A.P. P., R.A.P., C.B., B.L.M. Writing – review & editing: all authors.

Funding

This study was supported by grants from the Heart Foundation of New Zealand (HFNZ1936) and the Maurice & Phyllis Paykel Trust (221105) to BRP, RAP and JCMA.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Human ethics and consent to participate

The CDCS study was approved by the New Zealand (NZ) Multi-Region Ethics Committee, retrospectively registered at the Australian New Zealand Clinical Trials Registry (ACTRN12605000431628 on 16 September 2005), and all participating patients provided written, informed consent. The HVOL study was approved by the New Zealand Health and Disability Ethics Committee (Reference CTY/01/05/062) and registered with the Australian New Zealand Clinical Trials Registry (ACTRN1260500448640). All participating patients provided written, informed consent. Both studies conform to the principles outlined in the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46.

Consent for publication.

Not applicable – this article does not report an individual participant's data in any form.

Competing interests

B.R.P. is a Senior Editorial Board Member of BMC Cardiovascular Disorders.

Author details

[1]School of Health Sciences, School of Health Sciences, Massey University, Wellington, New Zealand
[2]The Christchurch Heart Institute, University of Otago, Christchurch, New Zealand
[3]Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

Received: 2 September 2024 / Accepted: 9 April 2025
Published online: 17 April 2025

References

1. Wilson N, et al. Prioritization of intervention domains to prevent cardiovascular disease: a country-level case study using global burden of disease and local data. Popul Health Metrics. 2023;21(1):1.

2. Roberts R, Chang CC, Hadley T. Genetic risk stratification: A paradigm shift in prevention of coronary artery disease. J Am Coll Cardiology: Basic Translational Sci. 2021;6(3):287–304.
3. Marks ECA, et al. Plasma levels of soluble VEGF receptor isoforms, Circulating pterins and VEGF system SNPs as prognostic biomarkers in patients with acute coronary syndromes. BMC Cardiovasc Disord. 2018;18(1):169.
4. Palmer BR, et al. Vascular endothelial growth factor-A promoter polymorphisms, Circulating VEGF-A and survival in acute coronary syndromes. PLoS ONE. 2021;16(7):1–15.
5. Zhou Y, et al. The role of the VEGF family in coronary heart disease. Front Cardiovasc Med. 2021;8:1–16.
6. Camaré C, et al. Angiogenesis in the atherosclerotic plaque. Redox Biol. 2017;12:18–34.
7. Niklander S, et al. Vascular endothelial growth factor: A translational view in oral Non-Communicable diseases. Biomolecules. 2021;11(1):1–27.
8. Shaik F, et al. Structural basis for vascular endothelial growth factor receptor activation and implications for disease therapy. Biomolecules. 2020;10(12):1–25.
9. Oszajca K, et al. Single-nucleotide polymorphisms of VEGF-A and VEGFR-2 genes and risk of infantile hemangioma. Int J Dermatol. 2018;57(10):1201–7.
10. Apte RS, Chen DS, Ferrara N. VEGF in signaling and disease: beyond discovery and development. Cell. 2019;176(6):1248–64.
11. Le THV, Kwon SM. Vascular endothelial growth factor biology and its potential as a therapeutic target in rheumatic diseases. Int J Mol Sci. 2021;22(10):1–17.
12. Peach CJ, et al. Molecular Pharmacology of VEGF-A isoforms: binding and signalling at VEGFR2. Int J Mol Sci. 2018;19(4):1264.
13. Smith GA, et al. Vascular endothelial growth factors: multitasking functionality in metabolism, health and disease. J Inherit Metab Dis. 2015;38(4):753–63.
14. Braile M, et al. VEGF-A in cardiomyocytes and heart diseases. Int J Mol Sci. 2020;21(15):1–18.
15. Au Yeung SL, Lam H, Schooling CM. Vascular endothelial growth factor and ischemic heart disease risk: A Mendelian randomization study. J Am Heart Association. 2017;6(8):1–8.
16. Zhang Y, et al. Serum vascular endothelial growth factor as a biomarker for prognosis of minor ischemic stroke. Clin Neurol Neurosurg. 2020;196:106060.
17. Rodríguez-Rodríguez L, et al. Vascular endothelial growth factor A and cardiovascular disease in rheumatoid arthritis patients. Tissue Antigens. 2011;77(4):291–7.
18. Heinonen SE, et al. The effects of VEGF-A on atherosclerosis, lipoprotein profile, and lipoprotein lipase in hyperlipidaemic mouse models. Cardiovascular Res. 2013;99(4):716–23.
19. Zhao Z, et al. Association between single nucleotide polymorphisms in cardiovascular developmental critical genes and hypertension: A propensity score matching analysis. Int J Hypertens. 2020;2020:1–8.
20. Ruotsalainen SE, et al. An expanded analysis framework for multivariate GWAS connects inflammatory biomarkers to functional variants and disease. Eur J Hum Genet. 2021;29(2):309–24.
21. Zhao X, et al. Vascular endothelial growth factor gene polymorphisms and coronary heart disease: a systematic review and meta-analysis. Growth Factors. 2018;36(3–4):153–63.
22. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. J Neuroimmune Pharmacol. 2014;9(2):142–60.
23. Pagès G, Pouysségur J. Transcriptional regulation of the vascular endothelial growth factor gene–a concert of activating factors. Cardiovascular Res. 2005;65(3):564–73.
24. Tischer E, et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J Biol Chem. 1991;266(18):11947–54.
25. Singh P, et al. Genetic scores of eNOS, ACE and VEGFA genes are predictive of endothelial dysfunction associated osteoporosis in postmenopausal women. Int J Environ Res Public Health. 2021;18(3):1–18.
26. Debette S, et al. Identification of cis- and trans-acting genetic variants explaining up to half the variation in Circulating vascular endothelial growth factor levels. Circul Res. 2011;109(5):554–63.
27. Choi SH, et al. Six novel loci associated with Circulating VEGF levels identified by a Meta-analysis of Genome-Wide association studies. Public Libr Sci (PLOS) Genet. 2016;12(2):e1005874.
28. Chen H, et al. Variation in VEGFA and risk of cardiovascular disease in the UK biobank. Front Cardiovasc Med. 2023;10:1240288.
29. Scott XO et al. Cohort study on the differential expression of inflammatory and angiogenic factors in thrombi, cerebral and peripheral plasma following acute large vessel occlusion stroke. J Cereb Blood Flow Metabolism, 2022: p. 271678x221106956.
30. Palmer BR, et al. KCNE5 polymorphism rs697829 is associated with QT interval and survival in acute coronary syndromes patients. J Cardiovasc Electrophys. 2012;23(3):319–24.
31. Palmer BR, et al. Angiotensin-converting enzyme 2 A1075G polymorphism is associated with survival in an acute coronary syndromes cohort. Am Heart J. 2008;156(4):752–8.
32. Ellis KL, et al. Genomic risk variants at 1p13.3, 1q41, and 3q22.3 are associated with subsequent cardiovascular outcomes in healthy controls and in established coronary artery disease. Circulation Cardiovasc Genet. 2011;4(6):636–46.
33. Lassé M, et al. Fibrinogen and hemoglobin predict near future cardiovascular events in asymptomatic individuals. Sci Rep. 2021;11(1):4605.
34. Palmer BR, et al. Genetic polymorphism rs6922269 in the MTHFD1L gene is associated with survival and baseline active vitamin B12 levels in post-acute coronary syndromes patients. PLoS ONE. 2014;9(3):e89029.
35. Prickett TC, et al. C-Type natriuretic peptides in coronary disease. Clin Chem. 2017;63(1):316–24.
36. Braunwald E, et al. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction. A report of the American college of cardiology/american heart association task force on practice guidelines (Committee on the management of patients with unstable Angina). J Am Coll Cardiol. 2000;36(3):970–1062.
37. Yadav BK, et al. Genetic polymorphisms rs699947, rs1570360, and rs3025039 on the VEGF gene are correlated with extracranial internal carotid artery stenosis and ischemic stroke. Ann Clin Lab Sci. 2017;47(2):144–55.
38. Skrypnik D, et al. Association of rs699947 (-2578 C/A) and rs2010963 (-634 G/C) single nucleotide polymorphisms of the VEGF gene, VEGF-A and leptin serum level, and cardiovascular risk in patients with excess body mass: A Case-Control study. J Clin Med. 2020;9(2):1–16.
39. Totomoch-Serra A, et al. Association of common polymorphisms in the and genes with type 2 diabetes-related traits in Mexicans. Archives Med Sci. 2018;14(6):1361–73.
40. Stathopoulou MG, et al. A common variant highly associated with plasma VEGFA levels also contributes to the variation of both LDL-C and HDL-C. J Lipid Res. 2013;54(2):535–41.
41. Sellami N, et al. Association of VEGFA variants with altered VEGF secretion and type 2 diabetes: A case-control study. Cytokine. 2018;106:29–34.
42. Nia SK et al. The impact of vascular endothelial growth factor + 405 C/G polymorphism on long-term outcome and severity of coronary artery disease. J Clin Lab Anal, 2017. 31(4).
43. Li L, et al. Association of genetic polymorphisms on vascular endothelial growth factor and its receptor genes with susceptibility to coronary heart disease. Med Sci Monit. 2016;22:31–40.
44. Ma W-Q, et al. Association of genetic polymorphisms in vascular endothelial growth factor with susceptibility to coronary artery disease: a meta–analysis. BMC Med Genet. 2018;19(1):1–12.
45. Wang W, Xu A, Xu H. The roles of vascular endothelial growth factor gene polymorphisms in congenital heart diseases: a meta-analysis. Growth Factors. 2018;36(5–6):232–8.
46. Meza-Alvarado JC et al. VEGF-A related SNPs: a cardiovascular context. Front Cardiovasc Med, 2023. 10.
47. Kent WJ, et al. The human genome browser at UCSC. Genome Res. 2002;12(6):996–1006.
48. Martin FJ, et al. Ensembl 2023. Nucleic Acids Res. 2022;51(D1):D933–41.
49. Das S, et al. Next-generation genotype imputation service and methods. Nat Genet. 2016;48(10):1284–7.
50. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. Nucleic Acids Res. 2016;44(D1):D877–81.
51. Rauluseviciute I, et al. JASPAR 2024: 20th anniversary of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2023;52(D1):D174–82.
52. Ghazizadeh H, et al. Association of rs6921438 A < G with serum vascular endothelial growth factor concentrations in patients with metabolic syndrome. Gene. 2018;667:70–5.
53. Ghazizadeh H, et al. Interaction between a genetic variant in vascular endothelial growth factor with dietary intakes in association with the main factors of metabolic syndrome. Gene Rep. 2020;21:100813.

54. Sliz E, et al. Genome-wide association study identifies seven novel loci associating with Circulating cytokines and cell adhesion molecules in Finns. J Med Genet. 2019;56(9):607–16.
55. Wang Y, et al. Genome-wide association study identifies 16 genomic regions associated with Circulating cytokines at birth. PLoS Genet. 2020;16(11):e1009163.
56. Ryu JR, et al. Stroke-associated intergenic variants modulate a human FOXF2 transcriptional enhancer. Proc Natl Acad Sci United States Am (USA). 2022;119(35):e2121333119.
57. Wu Q, Li W, You C. The regulatory roles and mechanisms of the transcription factor FOXF2 in human diseases. PeerJ. 2021;9:e10845.
58. Fahey E, Doyle SL. IL-1 family cytokine regulation of vascular permeability and angiogenesis. Front Immunol. 2019;10:1–15.
59. Ramji DP, Davies TS. Cytokines in atherosclerosis: key players in all stages of disease and promising therapeutic targets. Cytokine Growth Factor Rev. 2015;26(6):673–85.
60. Azimi-Nezhad M, et al. Associations of vascular endothelial growth factor (VEGF) with adhesion and inflammation molecules in a healthy population. Cytokine. 2013;61(2):602–7.
61. Harhous Z, et al. An update on the multifaceted roles of STAT3 in the heart. Front Cardiovasc Med. 2019;6:150.
62. Innocenti F, et al. Genetic variation determines VEGF-A plasma levels in cancer patients. Sci Rep. 2018;8(1):16332.
63. Zhang P, et al. The mechanism of the NFAT transcription factor family involved in oxidative stress response. J Cardiol. 2024;83(1):30–6.
64. Merlo S, et al. Vascular endothelial growth factor gene polymorphism (rs2010963) and its receptor, kinase insert Domain-Containing receptor gene polymorphism (rs2071559), and markers of carotid atherosclerosis in patients with type 2 diabetes mellitus. J Diabetes Res. 2016;2016:1–6.
65. Liu D, et al. Association of genetic polymorphisms on VEGFA and VEGFR2 with risk of coronary heart disease. Medicine. 2016;95(19):e3413.
66. O'Donnell C, et al. N-Terminal Pro-B-Type natriuretic peptide as a biomarker for the severity and outcomes with COVID-19 in a nationwide hospitalized cohort. J Am Heart Association. 2021;10(24):e022913.
67. Tzeng HE, et al. Interleukin-6 induces vascular endothelial growth factor expression and promotes angiogenesis through apoptosis signal-regulating kinase 1 in human osteosarcoma. Biochem Pharmacol. 2013;85(4):531–40.

Publisher's note