Supplemental Material

Identification of Cis- and Trans-acting Genetic Variants Explaining up to Half the Heritability of Circulating VEGF levels

A. Supplemental Methods

1. Cohorts

Framingham Heart Study (FHS)
The FHS is a three-generation, single-site, community-based, prospective cohort study that was initiated in 1948 to investigate risk factors for cardiovascular disease including stroke in Framingham, MA, USA. It now comprises 3 generations of participants: the original cohort followed since 1948 (Original, Gen 1)[1]; their offspring and spouses of the offspring, followed since 1971 (Offspring, Gen 2);[2] and grandchildren of the Original cohort in 2002 (Gen 3).[3] Vascular endothelial growth factor (VEGF) levels have at this time only been measured in Gen 3 participants. Starting in 2002, 4,095 participants with at least one parent in the Offspring cohort were enrolled in the Gen 3 cohort.[3] At their first examination (2002-2005) Gen 3 participants underwent a targeted medical history, physical examination, anthropometry, and laboratory assessment of traditional cardiovascular risk factors. VEGF levels were available in 3,946 participants, among whom we excluded persons with prevalent cardiovascular disease (n=66), serum creatinine >2 mg/dl (n=1) and those in whom data for more covariates (n=125) were not available. Of the remaining 3,754 participants, 3,553 also had DNA extracted and genome-wide genotyping data available. Genome-wide genotyping was performed at Affymetrix (Santa Clara, CA) through an NHLBI funded SNP-Health Association Resource (SHARe) project and was successful in 3,527 persons. These 3,527 persons constitute the FHS sample for this study.
All study participants provided informed consent and the study was approved by the Institutional Review Board at the Boston University Medical Center.

STANISLAS Family Study (SFS)
Initiated in 1993, the STANISLAS Family Study is a 10-year longitudinal survey set up to seek out gene-gene and gene-environment interactions in the field of cardiovascular diseases.[4] 1,006 volunteer families (2 parents and at least two siblings) were initially recruited between 1993 and 1995 during a free health check-up at the Centre for Preventive Medicine in Vandoeuvre-lès-Nancy, France. Individuals with chronic disorders (cardiovascular or cancer) or having a personal history of cardiovascular disease were not included, in order to assess the effects of genetic susceptibility factors on the variability of intermediate phenotypes in physiological conditions without the influence of any long term medication and disease. Biological and clinical measurements, health and lifestyle information were thus collected from 4,488 individuals belonging to 2 generations using appropriate, validated questionnaires and procedures as described previously.[4, 5] DNA was extracted from all participants and serum/plasma/DNA biobanks were constructed. In 1998–2000, 756 of the original families attended a second check-up and the same examination and biobanking protocols undertaken at the first examination were repeated. Using the plasma collected at this examination, specific measurements like VEGF plasma concentrations were measured on a random subsample of 1,000 subjects. A third check-up took place between 2003 and 2005 and at this visit mRNA and lymphocytes extract biobanks were constructed in addition to serum/plasma/DNA biobanks. Individuals with both VEGF plasma concentrations and DNA available were selected for the present study (919 individuals from 263 families). Some parents or children were secondarily excluded because DNA amplification was not successful (n=58), or results of genotyping were ambiguous (n=2). Finally, 859 individuals from 217 families met our selection criteria. Each subject gave written informed consent for participating in this study, which was approved by the Local Ethics Committee of Nancy.

Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study
All 70-year old individuals living in the community of Uppsala, Sweden, between April 2001 and June 2004 were eligible for the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study, which

has been described in detail previously.[6] The individuals were selected randomly, and were examined within one month of their 70th birthday in order to standardize for age. Of 2,025 individuals invited, 1,016 were investigated giving a participation rate of 50%, and 999 of these individuals provided DNA for genetic studies. For the purpose of the present investigation, we excluded participants with sample call rate <90% (n=11), prevalent myocardial infarction, stroke or congestive heart failure (n=116), serum creatinine >2 mg/dl (n=1), or missing data on VEGF (n=3). Thus, 868 participants (52% women) were eligible for the present study. The PIVUS study has been approved by the Ethics Committee of Uppsala University and the participants gave written informed consent.

2. Laboratory measurements of vascular endothelial growth factor

VEGF levels were measured in serum for the FHS and PIVUS and plasma for the SFS, at the first examination cycle (2002-2005) in the FHS, the second examination in the SFS (1998-2000) and the baseline examination (2001-2004) in the PIVUS study.
In all 3 studies venous blood samples were drawn after an overnight fast, immediately centrifuged and stored appropriately (at -80°C in FHS and PIVUS and at -196°C in liquid nitrogen in SFS) until VEGF measurements were undertaken. At FHS, serum VEGF was measured using a commercial ELISA assay (R&D Inc.). In SFS and PIVUS plasma VEGF and serum VEGF quantification respectively was performed by Randox Ltd (Crumlin, UK), using a biochip array analyzer (Evidence®).[7, 8] In all studies both diffusible VEGF isoforms ($VEGF_{121}$ and $VEGF_{165}$) were detected.
The average inter-assay coefficients of variation were 2.1% for serum VEGF in the FHS, less than 9% in the SFS and less than 15% in the PIVUS study.
Since serum VEGF had been measured in the FHS and PIVUS and plasma for VEGF in the SFS, we checked the correlation between the 2 types of specimens. VEGF was measured in a subset (n=18) of matched plasma and serum samples from the SFS. Plasma VEGF was lower (42±28 ng/L, mean±SD) than serum VEGF (361±223 ng/L); There was a strong correlation between plasma and serum VEGF (r=0.76, p=0.0002) which strengthens our study.

3. Genotyping, quality controls, and imputation

Genome-wide genotyping in the FHS study was performed on the Affymetrix GeneChip Human Mapping 500K Array Set® and 50K Human Gene Focused Panel®.
The set of genotyped input SNPs used for imputation was selected based on their highest quality GWA data. From a total of 534,982 genotyped autosomal SNPs in FHS, we used 378,163 SNPs in the imputation after filtering out 15,586 SNPs for Hardy-Weinberg disequilibrium ($p<1x10^{-6}$), 64,511 SNPs for missingness >0.03, 45,361 SNPs for a test of differential missingness yielding a $p<1x10^{-9}$ (mishap test in PLINK, http://pngu.mgh.harvard.edu/purcell/plink/), 4,857 SNPs for >100 Mendel errors, 67,269 SNPs for a minor allele frequency < 0.01, 2 SNPs due to strandedness issues upon merging data with HapMap, and a further 13,394 SNPs because they were not present on HapMap. We used the Markov Chain Haplotyping (MaCH) package (http://www.sph.umich.edu/csg/abecasis/MACH, version 1.0.15 software) and imputed to the plus strand of NCBI build 36, HapMap release #22. For each imputed SNP, imputation quality was estimated as the ratio of the empirically observed dosage variance to the expected binomial dosage variance. After quality control and filtering, FHS had either genotyped or imputed data for 2,540,223 autosomal SNPs. From a total of 10,886 genotyped SNPs on the X chromosome, we used 7,795 SNPs in the imputation after filtering out 3,091 SNPs for Hardy-Weinberg $p<1x10^{-6}$ (n=159), missingness >0.03 (n=450), minor allele frequency < 0.01 (n=1851), male heterozygote count > 45 (n=12), and a further 619 SNPs because they were not present on HapMap. We used the IMPUTE package (https://mathgen.stats.ox.ac.uk/impute/impute.html, version 0.5.0) and imputed to the plus strand of NCBI Build 35, Hapmap release #21.

4. Screening for latent population substructure

FHS was screened for latent population substructure, including cryptic relatedness, using EIGENSTRAT.[9, 10] We used 882 unrelated individuals to infer eigenvectors, then projected the rest of the subjects onto these eigenvectors. Principal components associated with the outcome were handled like any associated covariate that needs to be adjusted for. We have systematically tested the association of the ten first principal

components with VEGF serum levels and found that only the ninth principal component was significantly associated with VEGF serum levels. Therefore only this principal component was included as a covariate. in the GWAS.

5. Covariate definition

In all 3 studies, hypertension was defined as systolic blood pressure ≥140, diastolic blood pressure ≥90 or use of anti-hypertensive treatment and smokers were identified based on current smoking status. Standing waist circumference (WC) was obtained at the level of the umbilicus and central obesity was defined as a WC ≥102 cm in men and ≥89 cm in women. Metabolic syndrome was defined as having any 3 of the following traits: (i) central obesity, (ii) hypertension, (iii) hyperglycemia defined as fasting glucose ≥100 mg/dL or use of anti-diabetic medications or insulin, (iv) hypertriglyceridemia defined as serum or plasma triglycerides ≥150 mg/dL or being on any lipid-lowering medication, (v) low HDL cholesterol (<40 mg/d in men and <50 mg/d in women).[11] These covariates were measured at the same time as VEGF levels, at the first Gen 3 examination cycle (2002-2005) in the FHS, at the second examination in the SFS (1998-2000) and at the baseline examination (2001-2004) in the PIVUS study.

6. Selection of SNPs for replication

From all SNPs associated with VEGF levels at a p-value < $5x10^{-8}$ in the discovery cohort, we excluded SNPs with minor allele frequencies (MAF) <0.05 as well as those imputed SNPs with low imputation quality (ratio of the empirically observed dosage variance to the expected binomial dosage variance <0.6). The remaining SNPs were grouped by "bins", each bin comprising SNPs that are in very strong linkage disequilibrium (LD) with each other, i.e. with an r^2>0.8 with the most significant SNP in the bin. Within each bin we selected one SNP for replication (except for the bin with the most significant associations where 2 SNPs were selected). Typically, we selected the SNP with the lowest p-value within each bin. In 6 bins a SNP with a slightly higher p-value was chosen either because this SNP had been directly genotyped in the discovery cohort, whereas the SNP with the lowest p-value in the bin had been imputed (rs1776721 and rs1886979) or because there were stronger arguments for functionality for the SNP with the slightly higher p-value (rs16873291, rs1349319, rs6475920 and rs10967492).

7. Meta-analysis

For the meta-analysis of results from the FHS and the PIVUS study, which both measured VEGF levels in the serum, we used a fixed effects inverse-variance weighted meta-analysis technique. Beta estimates were weighted by their inverse variance and a combined estimate was obtained by summing the weighted betas and dividing by the summed weights.
For the meta-analysis of results from all three studies (FHS, SFS and PIVUS study), an effective sample size weighted meta-analysis technique was used, to account for the fact that VEGF levels were measured in the serum in the FHS and PIVUS study and in the plasma in the SFS. This method consists of combining each study's Z-statistic, weighted by their sample size. Imputed SNPs are weighted for information content. Importantly this method does not require studies to use the same measurement scale. We do acknowledge that differences between plasma and serum levels due to differences in the various blood components that contribute to these levels cannot be addressed using the sample size weighted meta-analysis method. Hence the genetic association results for SFS alone are also presented.
Prior to meta-analysis, strand alignment was verified across all studies. After meta-analysis, the genomic control parameter was calculated and used to remove any residual population-stratification. We undertook the meta-analyses at FHS using the METAL software (http://www.sph.umich.edu/csg/abecasis/Metal/index.html).

8. Genetic score calculation

Step 1:
We selected SNPs with independent effects by running a conditional GWAS. This was done in a forward stepwise fashion. First we ran a GWAS adjusting for age, gender, the ninth principal component and the most significantly associated SNP (rs6921438). We then ran a GWAS additionally adjusting for the most

significantly associated SNP in the aforementioned conditional GWAS. This process was repeated, by adding the most significantly associated SNP as a new covariate to the regression model, until all SNPs independently associated with VEGF level at a p-value < $5x10^{-8}$ were selected. All selected independent SNPs with a minor allele frequency > 5% were used to compute the genetic score (**Supplementary Table I**). In the PIVUS study, as rs6921438 genotypes were not available, rs4513773 was used instead to compute the genetic score (r^2 with rs6921438 = 0.90)

Step 2:
Genotypes for the SNPs selected in step 1 were coded as 0, 1 or 2 for genotyped SNPs (according to the number of minor alleles) and the imputed allele dosage was used for imputed SNPs.
To compute the genetic score the genotype value was weighted by the effect size estimate from the GWAS (**Supplementary Table I**):
Risk Score = SNP1_estimate * SNP1_genotype + SNP2_estimate * SNP2_genotype + SNP3_estimate * SNP3_genotype + SNP4_estimate * SNP4_genotype

Step 3:
We estimated the proportion of phenotype variance explained by each SNP incorporated in the risk score (h^2_q see Supplementary Table I): h^2_q was computed as follows, using the GWAF R-package:[12]

$$h_q^2 = \max\left(0, \frac{\sigma^2_{G.null} + \sigma^2_{e.null} - \sigma^2_{G.full} - \sigma^2_{e.full}}{Var(y)}\right)$$

Where:
Var(y)= total phenotypic variance,
$\sigma^2_{G.null}$ and $\sigma^2_{e.null}$ = the polygenic variance and error variance when modeling without the tested SNP, $\sigma^2_{G.full}$ and $\sigma^2_{e.full}$ = the polygenic variance and error variance when modeling with the tested SNP.

9. Biological pathway analysis

Genes located close to associated SNPs were investigated for relevant networks by the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, www.ingenuity.com). To build networks, IPA queries the Ingenuity Pathways Knowledge Base for biological interactions between identified "focus genes", in this case genes close to SNPs significantly associated with circulating VEGF levels in the GWAS, and all other gene objects stored in the knowledge base. It then generates a set of networks with a maximum network size of 35 genes. An underlying assumption is that highly-interconnected networks are likely to represent significant biological function,[13] thus IPA optimizes for triangular relationships between genes, favoring denser networks over more sparsely connected ones. Networks are displayed graphically as "nodes" (corresponding to genes or gene products) and the biological relationships between the nodes, referred to as "edges". IPA also computes a score, representing the $-\log_{10}$(p-value), where the p-value is the probability of finding f or more focus genes in a set of n genes randomly selected. If there are n genes in the network and f of them are focus genes, the p-value is the probability of finding f or more focus genes in a set of n genes randomly selected from the Global Molecular Network. It is calculated using Fisher's exact test.

10. VEGF gene expression analysis in peripheral blood mononuclear cells (PBMCs)

Sample preparation and quantification of the messenger RNA (mRNA) VEGF spliced forms
Two-hundred and twenty samples from the SFS were randomly selected for inclusion in the PBMCs transcriptomic study. Fresh whole blood (10mL) was collected by standardized venipuncture in EDTA tubes (Vacutainer™; Becton Dickinson, NJ, USA). PBMCs were isolated by centrifugation on a density gradient of Ficoll (Ficoll-Paque™ PLUS; Amersham BioSciences) and stored at -80°c until RNA extraction according to a well-validated protocol,[14] with high recovery of lymphocyte(97.5%). Total RNA was subsequently extracted with the MagNaPure automate, using the MagNA Pure LC RNA HP isolation kit and RNA HP Blood External lysis protocol (Roche Diagnostics, France). Reverse transcription of total RNA was performed using 200 units of M-MuLV Reverse Transcriptase with 0.25 µg of oligos(dt) (Promega, France) according to a previous described protocol.[14] Quantification of the transcripts coding for the $VEGF_{121}$ and $VEGF_{165}$ isoforms, and the beta 2 microglobulin (B2M) control gene, was performed

using TaqMan® and LightCycler technologies (LC TaqMan Master kitRoche Diagnostics, France). All experiments were performed in duplicate. The detection level for each transcript was between 1 and 10 copies for both transcripts and for B2M. RT-PCR optimization and specificity of RT-PCR products were examined using SYBR® Green technology (LC FastStart DNA Master^{PLUS} SYBR Green I kit, Roche Diagnostic, France), melting curves analysis and agarose gel electrophoresis of the PCR amplicons. Primers and probes were designed to specifically amplify the spliced forms of VEGF based on their splicing sites[15] with specific reverse primers or hydrolyzation probes spanning the variant specific exon boundaries, which also avoids amplification of contaminating genomic DNA. Hydrolyzation probes were labeled with the reporter dye FAM (6-carboxy-fluorescein phosphoramidite) at the 5' end and the quencher dye TAMRA (5 -carboxyl- tetramethyl-rhodamine) at the 3' end. For all assays, intra- and inter-run variability were 11% and 5% respectively. PCR products for each VEGF mRNA spliced variant amplification were purified with a PCR purification kit (QiaQuick, Qiagen, France). The product concentrations were measured in a spectrophotometer, the molecule concentrations were calculated, and a standard curve was generated for each transcript using serial dilutions of products ranging from 1 or 10 to 10^7 molecules/μL. The copy number of unknown samples was calculated by setting their PCR cycle number (Crossing Point: CP) to the standard curve and normalized to the housekeeping B2M gene. Results are presented as copies of the target gene product per 10^6 copies of B2M. Primer efficiencies were calculated according to the equation $E = 10[-1/slope]$. All investigated transcripts had real-time PCR efficiency rates above 1.9.

PBMCs VEGF protein measurements

PBMC VEGF (121 and 165) protein quantification was performed by Randox Ltd (Crumlin, UK), with a biochip array analyzer (Evidence®) using a high sensitivity kit as previously described.[7] PBMC VEGF concentrations were log10-transformed in all analyses in order to improve normality, were adjusted for the effect of between-run variation and regressed on mean values of all samples measured in each run. Grubbs' test was applied for detection of the extreme values in the data (log transformed VEGF) and there were no outliers at the 5% level. The average inter-assay coefficient of variation was 5.7%.

Statistical analysis

A linear mixed effects model that accounts for within family correlation was used to evaluate the association of each of the SNPs successfully genotyped in the SFS with each of the two transcript levels ($VEGF_{121}$ and $VEGF_{165}$) and with natural log-transformed PBMC VEGF concentration, assuming an additive genetic model. These analyses were adjusted for age and sex.

11. Candidate gene association studies

We searched Pubmed for original articles published before May 14 2010, which reported statistically significant associations between specific genotypes and circulating VEGF levels. We used the search terms VEGF, gene and polymorphism. We identified 14 candidate gene association studies,[16-29] who examined 11 different polymorphisms in the VEGF gene in association with plasma or serum VEGF levels (**Supplementary Table IV**). Of these, 8 studies reported a significant association of at least one polymorphism (rs699947, rs1570360, rs833061, rs2010963, rs3025039, -2549 18bp I/D) with circulating VEGF levels.[22-29] In the FHS sample we replicated the association of rs699947 and rs833061 with serum VEGF levels at $p < 5x10^{-7}$ (**Supplementary Table IV**). The other 4 polymorphisms (rs1570360, rs2010963, rs3025039, -2549 18bp I/D) had neither been genotyped nor imputed in the FHS GWAS. As these SNPs are not in the Hapmap database, we could not identify any proxy either to test their association with serum VEGF levels in the FHS dataset.

B. Supplemental Tables

Online Table I: Results of the main and conditional GWAS within the Framingham Heart Study sample for the SNPs retained in the genetic score calculation

SNP	chr	position	CA	CAF	Estimate in main GWAS*	SE in main GWAS*	p in main GWAS*	p in conditional GWAS†	h^2_q (%)
rs6921438	6	44033585	G	0.51	0.7199	0.0149	$<5x10^{-324}$	$<5x10^{-324}$	41.19
rs10738760	9	2681186	A	0.49	0.2812	0.0230	$1.96x10^{-34}$	$3.78x10^{-47}$	4.97
rs6993770	8	106650704	T	0.32	-0.1667	0.0203	$2.50x10^{-16}$	$5.45x10^{-30}$	2.03
rs4416670	6	44058431	T	0.55	0.1342	0.0190	$1.47x10^{-12}$	$4.79x10^{-9}$	1.46

CA: coded allele; CAF: coded allele frequency; Chr: chromosome; GWAS: genome-wide association study; h^2_q: percentage of phenotypic variance explained; SE: standard error; * adjusted for age, sex, and the ninth principal component; † with all four SNPs in the same model, additionally adjusted for age, sex, and the ninth principal component

Online Table II: SNPs associated with serum VEGF levels at a p-value < $5x10^{-8}$ in the discovery GWAS

SNP	Chr	Position	CA	CAF	beta	SE	P	Gene 1	Distance	Gene 2	Distance	Function	O/E ratio	Imputed
rs6921438	6	44033585	G	0,52	0,72	0,01	6.11E-506	MGC45491	42731	MRPL14	155764	INTERGENIC	0,99	0
rs7767396	6	44035028	G	0,47	-0,71	0,02	1.71E-482	MGC45491	41288	MRPL14	154321	INTERGENIC	0,99	1
rs4513773	6	44033504	G	0,46	-0,71	0,02	2.08E-482	MGC45491	42812	MRPL14	155845	INTERGENIC	1,00	0
rs9472159	6	44027673	C	0,51	0,76	0,02	4.30E-452	MGC45491	48643	MRPL14	161676	INTERGENIC	0,82	1
rs9369434	6	44026385	C	0,53	0,85	0,02	2.15E-442	MGC45491	49931	MRPL14	162964	INTERGENIC	0,66	1
rs6916314	6	44027140	G	0,38	0,92	0,02	3.13E-400	MGC45491	49176	MRPL14	162209	INTERGENIC	0,54	1
rs9472158	6	44026875	G	0,38	0,92	0,02	6.22E-400	MGC45491	49441	MRPL14	162474	INTERGENIC	0,54	1
rs6916540	6	44027394	C	0,40	0,87	0,02	3.60E-364	MGC45491	48922	MRPL14	161955	INTERGENIC	0,56	1
rs9472173	6	44041234	C	0,47	0,93	0,02	1.16E-355	MGC45491	35082	MRPL14	148115	INTERGENIC	0,47	1
rs729391	6	44025870	C	0,39	1,05	0,03	7.17E-355	MGC45491	50446	MRPL14	163479	INTERGENIC	0,38	1
rs7764227	6	44021966	G	0,26	1,32	0,04	1,41E-228	MGC45491	54350	VEGFA	159767	INTERGENIC	0,22	1
rs9369433	6	44022528	C	0,23	1,28	0,04	6,87E-225	MGC45491	53788	VEGFA	160329	INTERGENIC	0,25	1
rs1359617	6	44024870	T	0,11	1,87	0,06	1,56E-194	MGC45491	51446	VEGFA	162671	INTERGENIC	0,18	1
rs865585	6	44019529	C	0,29	0,83	0,04	3,16E-125	MGC45491	56787	VEGFA	157330	INTERGENIC	0,30	1
rs6458360	6	44028870	T	0,11	1,10	0,05	3,14E-89	MGC45491	47446	MRPL14	160479	INTERGENIC	0,28	1
rs17209449	6	44043245	C	0,09	-1,20	0,06	1,30E-76	MGC45491	33071	MRPL14	146104	INTERGENIC	0,24	1
rs10738760	9	2681186	A	0,49	0,28	0,02	1,96E-34	KCNV2	26339	VLDLR	36701	INTERGENIC	0,68	1
rs10757631	9	2680295	G	0,47	-0,26	0,02	2,30E-32	KCNV2	27230	VLDLR	35810	INTERGENIC	0,73	1
rs6475919	9	2662809	T	0,37	-0,24	0,02	3,63E-32	VLDLR	18324	KCNV2	44716	INTERGENIC	0,95	1
rs6475920	9	2663933	A	0,37	-0,24	0,02	3,76E-32	VLDLR	19448	KCNV2	43592	INTERGENIC	0,96	1
rs1020651	9	2666360	G	0,37	-0,23	0,02	8,60E-32	VLDLR	21875	KCNV2	41165	INTERGENIC	1,01	1
rs1020652	9	2667012	T	0,37	-0,23	0,02	8,69E-32	VLDLR	22527	KCNV2	40513	INTERGENIC	1,01	0
rs10738758	9	2672355	G	0,37	-0,23	0,02	9,11E-32	VLDLR	27870	KCNV2	35170	INTERGENIC	0,98	1
rs4741756	9	2658187	C	0,28	-0,25	0,02	2,95E-31	VLDLR	13702	KCNV2	49338	INTERGENIC	0,97	0
rs2375980	9	2682622	G	0,42	-0,25	0,02	1,30E-27	KCNV2	24903	VLDLR	38137	INTERGENIC	0,70	1
rs7856084	9	2672943	C	0,39	-0,22	0,02	1,75E-27	VLDLR	28458	KCNV2	34582	INTERGENIC	0,97	1
rs10125071	9	2669579	C	0,40	-0,21	0,02	5,88E-27	VLDLR	25094	KCNV2	37946	INTERGENIC	1,00	1
rs4317630	9	2671025	C	0,39	-0,21	0,02	9,65E-27	VLDLR	26540	KCNV2	36500	INTERGENIC	0,98	1

rs10122587	9	2681951	T	0,28	-0,22	0,02	3,02E-24	KCNV2	25574	VLDLR	37466	INTERGENIC	0,98	1
rs9381273	6	44084246	G	0,01	5,92	0,59	5,37E-24	MGC45491	3381	MRPL14	105103	DOWNSTREAM	0,03	1
rs7747448	6	43990902	A	0,34	-0,25	0,03	6,88E-23	MGC45491	85414	VEGFA	128703	INTRONIC	0,58	1
rs4276495	6	43991237	T	0,32	-0,23	0,02	6,46E-22	MGC45491	85079	VEGFA	129038	INTRONIC	0,69	1
rs10117473	9	2670425	G	0,21	-0,22	0,02	7,67E-22	VLDLR	25940	KCNV2	37100	INTERGENIC	1,01	1
rs10967492	9	2671175	A	0,21	-0,22	0,02	1,02E-21	VLDLR	26690	KCNV2	36350	INTERGENIC	1,01	1
rs10967470	9	2665698	G	0,24	-0,22	0,02	1,17E-21	VLDLR	21213	KCNV2	41827	INTERGENIC	0,97	1
rs10812475	9	2681546	C	0,21	-0,22	0,02	1,84E-21	KCNV2	25979	VLDLR	37061	INTERGENIC	0,98	1
rs10812474	9	2681446	G	0,21	-0,22	0,02	1,86E-21	KCNV2	26079	VLDLR	36961	INTERGENIC	0,98	1
rs10812473	9	2681329	A	0,21	-0,22	0,02	1,86E-21	KCNV2	26196	VLDLR	36844	INTERGENIC	0,98	1
rs10812471	9	2680980	T	0,21	-0,22	0,02	1,89E-21	KCNV2	26545	VLDLR	36495	INTERGENIC	0,99	1
rs10125245	9	2679244	T	0,21	-0,22	0,02	2,09E-21	KCNV2	28281	VLDLR	34759	INTERGENIC	1,00	1
rs10967512	9	2673939	C	0,21	-0,22	0,02	2,18E-21	VLDLR	29454	KCNV2	33586	INTERGENIC	1,02	0
rs10757628	9	2674656	T	0,21	-0,22	0,02	2,22E-21	VLDLR	30171	KCNV2	32869	INTERGENIC	1,01	0
rs10812472	9	2681095	G	0,20	-0,23	0,02	4,89E-21	KCNV2	26430	VLDLR	36610	INTERGENIC	0,94	1
rs7867894	9	2665867	C	0,25	-0,21	0,02	8,62E-21	VLDLR	21382	KCNV2	41658	INTERGENIC	0,98	1
rs9472175	6	44042760	T	0,15	-0,56	0,06	9,73E-21	MGC45491	33556	MRPL14	146589	INTERGENIC	0,20	1
rs1776717	6	44059314	A	0,21	-0,23	0,02	8,10E-20	MGC45491	17002	MRPL14	130035	INTERGENIC	0,86	1
rs910609	6	44059634	A	0,21	-0,23	0,02	9,14E-20	MGC45491	16682	MRPL14	129715	INTERGENIC	0,86	1
rs833623	6	44006755	C	0,41	0,18	0,02	1,07E-19	MGC45491	69561	VEGFA	144556	INTRONIC	0,92	1
rs833622	6	44008382	T	0,40	0,17	0,02	1,21E-19	MGC45491	67934	VEGFA	146183	INTRONIC	0,98	0
rs1776721	6	43998961	T	0,30	-0,18	0,02	1,52E-19	MGC45491	77355	VEGFA	136762	INTRONIC	0,99	0
rs9381267	6	44013484	G	0,41	0,17	0,02	3,15E-19	MGC45491	62832	VEGFA	151285	3PRIME_UTR	0,97	1
rs1886979	6	44012879	G	0,41	0,17	0,02	3,72E-19	MGC45491	63437	VEGFA	150680	3PRIME_UTR	0,97	0
rs10122524	9	2681667	T	0,30	-0,19	0,02	3,73E-19	KCNV2	25858	VLDLR	37182	INTERGENIC	0,99	1
rs9472155	6	44005705	T	0,22	-0,20	0,02	4,45E-19	MGC45491	70611	VEGFA	143506	INTRONIC	0,96	1
rs7767550	6	44007230	A	0,22	-0,20	0,02	6,36E-19	MGC45491	69086	VEGFA	145031	INTRONIC	0,99	0
rs3888006	6	44000916	A	0,22	-0,20	0,02	7,60E-19	MGC45491	75400	VEGFA	138717	INTRONIC	0,95	1
rs1750570	6	44000213	T	0,22	-0,20	0,02	8,79E-19	MGC45491	76103	VEGFA	138014	INTRONIC	0,95	1
rs1326141	6	44011255	A	0,22	-0,20	0,02	2,43E-18	MGC45491	65061	VEGFA	149056	INTRONIC	0,98	0
rs910608	6	44059804	C	0,22	-0,21	0,02	3,39E-18	MGC45491	16512	MRPL14	129545	INTERGENIC	0,87	1
rs7356919	6	43999343	A	0,22	-0,20	0,02	5,37E-18	MGC45491	76973	VEGFA	137144	INTRONIC	0,96	1

rs910610	6	44059418	A	0,23	-0,21	0,03	2,50E-17	MGC45491	16898	MRPL14	129931	INTERGENIC	0,79	1
rs1418898	6	44027285	A	0,01	-2,60	0,31	7,27E-17	MGC45491	49031	MRPL14	162064	INTERGENIC	0,14	1
rs1418897	6	44027365	G	0,01	-2,60	0,31	7,37E-17	MGC45491	48951	MRPL14	161984	INTERGENIC	0,14	1
rs9296424	6	44043864	C	0,18	-0,49	0,06	9,98E-17	MGC45491	32452	MRPL14	145485	INTERGENIC	0,18	1
rs1631938	6	44060024	A	0,23	-0,20	0,02	1,74E-16	MGC45491	16292	MRPL14	129325	INTERGENIC	0,83	1
rs7761865	6	43976194	G	0,36	-0,51	0,06	2,21E-16	MGC45491	100122	VEGFA	113995	INTRONIC	0,10	1
rs6993770	8	106650704	T	0,32	-0,17	0,02	2,50E-16	ZFPM2	0	LRP12	980360	INTRONIC	0,97	1
rs9367181	6	44064593	G	0,20	-0,19	0,02	3,61E-16	MGC45491	11723	MRPL14	124756	INTERGENIC	0,98	0
rs1740074	6	44055768	G	0,06	-0,46	0,06	8,17E-16	MGC45491	20548	MRPL14	133581	INTERGENIC	0,44	1
rs11968152	6	43944908	A	0,04	1,16	0,14	1,18E-15	VEGFA	82709	MGC45491	131408	INTERGENIC	0,11	1
rs3932536	6	43944278	A	0,04	1,15	0,14	1,43E-15	VEGFA	82079	MGC45491	132038	INTERGENIC	0,11	1
rs6936668	6	43944048	C	0,04	1,15	0,14	1,45E-15	VEGFA	81849	MGC45491	132268	INTERGENIC	0,11	1
rs7832219	8	106648153	C	0,30	-0,16	0,02	1,72E-15	ZFPM2	0	LRP12	977809	INTRONIC	0,98	1
rs4734879	8	106652300	G	0,30	-0,16	0,02	1,78E-15	ZFPM2	0	LRP12	981956	INTRONIC	0,98	1
rs2343592	8	106641446	G	0,30	-0,16	0,02	1,98E-15	ZFPM2	0	LRP12	971102	INTRONIC	0,97	1
rs4734875	8	106623913	C	0,26	-0,16	0,02	9,46E-15	ZFPM2	0	LRP12	953569	INTRONIC	1,00	0
rs16873346	8	106618657	C	0,26	-0,16	0,02	1,07E-14	ZFPM2	0	LRP12	948313	INTRONIC	0,99	1
rs844294	6	44008685	C	0,51	-0,15	0,02	1,19E-14	MGC45491	67631	VEGFA	146486	INTRONIC	0,97	1
rs4236085	6	43971742	C	0,38	-0,43	0,06	1,27E-14	MGC45491	104574	VEGFA	109543	INTRONIC	0,12	1
rs16873402	8	106658423	T	0,33	-0,15	0,02	1,97E-14	ZFPM2	0	LRP12	988079	INTRONIC	0,99	1
rs910604	6	44061649	A	0,20	-0,18	0,02	2,15E-14	MGC45491	14667	MRPL14	127700	INTERGENIC	0,98	0
rs16873418	8	106661321	G	0,33	-0,15	0,02	2,28E-14	ZFPM2	0	LRP12	990977	INTRONIC	0,99	1
rs6931378	6	43982797	A	0,50	0,37	0,05	7,27E-14	MGC45491	93519	VEGFA	120598	INTRONIC	0,14	1
rs4416670	6	44058431	T	0,55	0,13	0,02	1,47E-12	MGC45491	17885	MRPL14	130918	INTERGENIC	0,99	0
rs1740077	6	44057885	C	0,56	0,13	0,02	5,21E-12	MGC45491	18431	MRPL14	131464	INTERGENIC	0,96	1
rs1776704	6	44057919	G	0,56	0,13	0,02	5,38E-12	MGC45491	18397	MRPL14	131430	INTERGENIC	0,96	1
rs16873365	8	106627411	T	0,21	-0,16	0,02	5,65E-12	ZFPM2	0	LRP12	957067	INTRONIC	0,92	1
rs1776706	6	44057965	C	0,56	0,13	0,02	5,79E-12	MGC45491	18351	MRPL14	131384	INTERGENIC	0,97	1
rs4320369	6	44058040	C	0,56	0,13	0,02	5,98E-12	MGC45491	18276	MRPL14	131309	INTERGENIC	0,97	1
rs1740079	6	44059217	T	0,56	0,13	0,02	6,02E-12	MGC45491	17099	MRPL14	130132	INTERGENIC	0,96	1
rs910612	6	44058684	T	0,44	-0,13	0,02	6,07E-12	MGC45491	17632	MRPL14	130665	INTERGENIC	0,97	1
rs910613	6	44058614	T	0,56	0,13	0,02	6,10E-12	MGC45491	17702	MRPL14	130735	INTERGENIC	0,97	1

rs910614	6	44058596	T	0,44	-0,13	0,02	6,12E-12	MGC45491	17720	MRPL14	130753	INTERGENIC	0,97	1
rs7013321	8	106662734	A	0,48	-0,14	0,02	6,75E-12	ZFPM2	0	LRP12	992390	INTRONIC	0,83	1
rs6993696	8	106650460	A	0,46	-0,13	0,02	8,54E-12	ZFPM2	0	LRP12	980116	INTRONIC	0,99	1
rs4741755	9	2657929	C	0,30	-0,23	0,03	2,26E-11	VLDLR	13444	KCNV2	49596	INTERGENIC	0,37	1
rs16873287	8	106597151	G	0,30	-0,13	0,02	5,13E-11	ZFPM2	0	LRP12	926807	INTRONIC	1,02	0
rs16873291	8	106597206	T	0,30	-0,13	0,02	5,30E-11	ZFPM2	0	LRP12	926862	INTRONIC	1,02	0
rs12676726	8	106597884	C	0,30	-0,13	0,02	5,44E-11	ZFPM2	0	LRP12	927540	INTRONIC	1,01	1
rs9472147	6	43944737	A	0,17	-0,49	0,07	5,78E-11	VEGFA	82538	MGC45491	131579	INTERGENIC	0,12	1
rs10105733	8	106595037	G	0,29	-0,14	0,02	6,09E-11	ZFPM2	0	LRP12	924693	INTRONIC	1,01	1
rs4734119	8	106599699	G	0,30	-0,13	0,02	7,33E-11	ZFPM2	0	LRP12	929355	INTRONIC	1,01	0
rs1157141	8	106600486	G	0,30	-0,13	0,02	7,33E-11	ZFPM2	0	LRP12	930142	INTRONIC	1,01	1
rs1157142	8	106600850	A	0,30	-0,13	0,02	7,34E-11	ZFPM2	0	LRP12	930506	INTRONIC	1,01	1
rs6996138	8	106601653	G	0,30	-0,13	0,02	7,35E-11	ZFPM2	0	LRP12	931309	INTRONIC	1,01	1
rs4734873	8	106602506	A	0,30	-0,13	0,02	7,36E-11	ZFPM2	0	LRP12	932162	INTRONIC	1,01	0
rs7001868	8	106602795	G	0,30	-0,13	0,02	7,38E-11	ZFPM2	0	LRP12	932451	INTRONIC	1,01	1
rs10094510	8	106592361	A	0,29	-0,13	0,02	8,33E-11	ZFPM2	0	LRP12	922017	INTRONIC	1,02	0
rs7007968	8	106591021	G	0,29	-0,13	0,02	9,27E-11	ZFPM2	0	LRP12	920677	INTRONIC	1,02	1
rs16873231	8	106587411	G	0,29	-0,13	0,02	1,36E-10	ZFPM2	0	LRP12	917067	INTRONIC	1,01	1
rs6997293	8	106587104	C	0,29	-0,13	0,02	1,47E-10	ZFPM2	0	LRP12	916760	INTRONIC	1,01	1
rs1868650	8	106587088	C	0,29	-0,13	0,02	1,52E-10	ZFPM2	0	LRP12	916744	INTRONIC	1,01	1
rs1868649	8	106587041	A	0,29	-0,13	0,02	1,57E-10	ZFPM2	0	LRP12	916697	INTRONIC	1,01	1
rs2291192	8	106586248	A	0,29	-0,13	0,02	1,77E-10	ZFPM2	0	LRP12	915904	INTRONIC	1,01	1
rs12678719	8	106585230	G	0,29	-0,13	0,02	1,95E-10	ZFPM2	0	LRP12	914886	INTRONIC	1,01	1
rs10093110	8	106634590	A	0,43	-0,12	0,02	2,18E-10	ZFPM2	0	LRP12	964246	INTRONIC	0,99	0
rs6988664	8	106582477	G	0,29	-0,13	0,02	2,33E-10	ZFPM2	0	LRP12	912133	INTRONIC	1,01	1
rs4734869	8	106582004	A	0,29	-0,13	0,02	2,60E-10	ZFPM2	0	LRP12	911660	INTRONIC	1,01	0
rs910611	6	44058829	C	0,08	-0,26	0,04	2,61E-10	MGC45491	17487	MRPL14	130520	INTERGENIC	0,77	1
rs748785	9	2684347	C	0,13	0,24	0,04	1,01E-09	KCNV2	23178	VLDLR	39862	INTERGENIC	0,52	1
rs7767854	6	44065304	T	0,11	-0,22	0,04	1,02E-09	MGC45491	11012	MRPL14	124045	INTERGENIC	0,74	1
rs6995272	8	106662838	T	0,46	0,14	0,02	1,98E-09	ZFPM2	0	LRP12	992494	INTRONIC	0,66	1
rs12675041	8	106598221	A	0,34	-0,12	0,02	2,10E-09	ZFPM2	0	LRP12	927877	INTRONIC	0,96	1
rs1740080	6	44060390	A	0,12	-0,18	0,03	2,76E-09	MGC45491	15926	MRPL14	128959	INTERGENIC	0,93	1

rs910606	6	44061307	A	0,12	-0,18	0,03	2,90E-09	MGC45491	15009	MRPL14	128042	INTERGENIC	0,94	0
rs1631662	6	44061336	T	0,12	-0,18	0,03	2,93E-09	MGC45491	14980	MRPL14	128013	INTERGENIC	0,94	1
rs2051074	6	44056431	T	0,04	-0,41	0,07	5,80E-09	MGC45491	19885	MRPL14	132918	INTERGENIC	0,46	1
rs9381262	6	43983427	A	0,45	-0,29	0,05	6,78E-09	MGC45491	92889	VEGFA	121228	INTRONIC	0,14	1
rs1450163	8	106601298	C	0,37	-0,11	0,02	1,11E-08	ZFPM2	0	LRP12	930954	INTRONIC	1,00	1
rs7836542	8	106603619	T	0,37	-0,11	0,02	1,13E-08	ZFPM2	0	LRP12	933275	INTRONIC	1,01	0
rs16897113	6	44076194	T	0,03	0,62	0,11	1,34E-08	MGC45491	122	MRPL14	113155	UPSTREAM	0,29	1
rs2343595	8	106660383	G	0,45	-0,11	0,02	2,26E-08	ZFPM2	0	LRP12	990039	INTRONIC	1,01	1
rs16873415	8	106660978	G	0,45	-0,11	0,02	2,39E-08	ZFPM2	0	LRP12	990634	INTRONIC	1,01	0
rs1901061	8	106619426	A	0,39	0,11	0,02	3,08E-08	ZFPM2	0	LRP12	949082	INTRONIC	0,94	1
rs1349319	8	106625810	A	0,39	0,11	0,02	3,59E-08	ZFPM2	0	LRP12	955466	INTRONIC	0,94	1
rs9369430	6	43942229	G	0,16	0,24	0,04	3,68E-08	VEGFA	80030	MGC45491	134087	INTERGENIC	0,33	1
rs748786	9	2684436	T	0,12	0,23	0,04	3,91E-08	KCNV2	23089	VLDLR	39951	INTERGENIC	0,52	1
rs4734122	8	106629546	A	0,41	0,11	0,02	4,15E-08	ZFPM2	0	LRP12	959202	INTRONIC	0,99	1
rs1375955	8	106624215	T	0,39	0,11	0,02	4,30E-08	ZFPM2	0	LRP12	953871	INTRONIC	0,94	1
rs12679049	8	106598022	G	0,22	-0,12	0,02	4,75E-08	ZFPM2	0	LRP12	927678	INTRONIC	0,99	0

CA: coded allele; CAF: coded allele frequency; Chr: chromosome; KCNV2: potassium voltage-gated channel subfamily V, member 2; LRP12: low-density lipoprotein receptor-related protein gene; MCG45491: uncharacterized protein, also known as C6orf223; MRPL14: mitochondrial ribosomal protein L14; O/E ratio: observed over expected ratio (measuring imputation accuracy); p: p-value; SE: standard error; SNP: single nucleotide polymorphism; VEGF: vascular endothelial growth factor; VLDLR: very low density lipoprotein receptor; ZFPM2: zinc finger protein, multitype 2

Online Table III: Test of heterogeneity between studies in the meta-analyses combining discovery and replication cohorts

SNP	Chr	$p_{heterogeneity}$	
		Inverse variance weighted meta-analysis (FHS+PIVUS)	Effective sample size weighted meta-analysis (FHS+SFS+PIVUS)
rs6921438	6	1	5.50×10^{-21}
rs4513773	6	0.52	0.19
rs9472159	6	5.70×10^{-3}	6.07×10^{-18}
rs9369434	6	4.32×10^{-15}	7.74×10^{-26}
rs1776717	6	0.18	0.69
rs1776721	6	0.31	0.068
rs1886979	6	0.90	0.20
rs9472155	6	0.20	0.056
rs844294	6	0.71	0.14
rs4416670	6	0.10	0.23
rs910611	6	0.52	0.13
rs6993770	8	0.087	0.099
rs16873402	8	0.033	0.011
rs16873365	8	0.052	0.022
rs7013321	8	1	0.41
rs6993696	8	0.67	0.39
rs16873291	8	0.069	0.098
rs1349319	8	0.66	0.70
rs10738760	9	0.14	2.20×10^{-3}
rs6475920	9	0.78	7.20×10^{-3}
rs4741756	9	0.11	9.84×10^{-4}
rs2375980	9	0.44	0.018
rs10122587	9	1	0.015
rs10967492	9	1	5.79×10^{-3}
rs10967470	9	1	0.016

$p_{heterogeneity}$: p-value for Cochran's Q-statistic for heterogeneity

Online Table IV: Secondary genetic association analysis adjusting for clinical covariates previously found to be associated with VEGF levels

SNPID	Chr	Position	p (FHS)	p (PIVUS)	p (SFS)	Meta-p (FHS+PIVUS) *	Meta-p (all) †
Model B							
rs6921438	6	44033585	$1.72x10^{-506}$	NA	$1.84x10^{-39}$	$1.72x10^{-506}$	$1.06x10^{-524}$
rs4513773	6	44033504	$1.58x10^{-482}$	$7.98x10^{-139}$	NA	$4.41x10^{-619}$	$1.28x10^{-584}$
rs9472159	6	44027673	$2.89x10^{-452}$	$2.90x10^{-109}$	$3.58x10^{-35}$	$8.76x10^{-558}$	$7.76x10^{-553}$
rs9369434	6	44026385	$8.70x10^{-443}$	$3.02x10^{-63}$	$5.81x10^{-28}$	$1.54x10^{-490}$	$2.23x10^{-496}$
rs1776717	6	44059314	$1.23x10^{-19}$	$2.27x10^{-4}$	$8.96x10^{-6}$	$2.59x10^{-22}$	$8.45x10^{-27}$
rs1776721	6	43998961	$1.17x10^{-19}$	$4.34x10^{-8}$	0.018	$5.02x10^{-26}$	$3.12x10^{-26}$
rs1886979	6	44012879	$3.23x10^{-19}$	$2.71x10^{-6}$	0.013	$4.82x10^{-24}$	$1.29x10^{-24}$
rs9472155	6	44005705	$4.98x10^{-19}$	$4.83x10^{-9}$	0.014	$3.39x10^{-26}$	$1.68x10^{-26}$
rs844294	6	44008685	$1.34x10^{-14}$	$1.95x10^{-5}$	0.087	$1.41x10^{-18}$	$2.50x10^{-18}$
rs4416670	6	44058431	$2.04x10^{-12}$	0.1	$2.79x10^{-4}$	$1.95x10^{-12}$	$2.81x10^{-15}$
rs910611	6	44058829	$4.77x10^{-10}$	$4.72x10^{-6}$	0.11	$1.43x10^{-14}$	$2.99x10^{-14}$
rs6993770	8	106650704	$2.03x10^{-16}$	$3.61x10^{-8}$	0.016	$1.94x10^{-22}$	$2.96x10^{-23}$
rs16873402	8	106658423	$1.45x10^{-14}$	$1.16x10^{-8}$	0.14	$9.09x10^{-21}$	$3.86x10^{-20}$
rs16873365	8	106627411	$6.93x10^{-12}$	$2.10x10^{-6}$	0.37	$5.14x10^{-16}$	$2.91x10^{-15}$
rs7013321	8	106662734	$4.66x10^{-12}$	NA	0.013	$4.66x10^{-12}$	$2.84x10^{-13}$
rs6993696	8	106650460	$8.83x10^{-12}$	$1.57x10^{-4}$	0.040	$6.67x10^{-15}$	$1.82x10^{-15}$
rs16873291	8	106597206	$5.43x10^{-11}$	$1.42x10^{-6}$	0.061	$1.76x10^{-15}$	$6.60x10^{-16}$
rs1349319	8	106625810	$2.93x10^{-8}$	$1.73x10^{-3}$	0.040	$2.03x10^{-10}$	$3.05x10^{-11}$
rs10738760	9	2681186	$1.17x10^{-34}$	$3.24x10^{-9}$	0.035	$6.36x10^{-42}$	$2.26x10^{-40}$
rs6475920	9	2663933	$2.23x10^{-32}$	$1.46x10^{-8}$	0.022	$2.21x10^{-39}$	$3.30x10^{-38}$
rs4741756	9	2658187	$2.29x10^{-31}$	$7.05x10^{-5}$	0.097	$2.73x10^{-34}$	$2.65x10^{-32}$
rs2375980	9	2682622	$1.53x10^{-27}$	$8.92x10^{-9}$	0.022	$1.10x10^{-34}$	$5.61x10^{-34}$
rs10122587	9	2681951	$3.89x10^{-24}$	NA	0.026	$3.89x10^{-24}$	$6.74x10^{-24}$
rs10967492	9	2671175	$1.86x10^{-21}$	NA	0.11	$1.86x10^{-21}$	$2.36x10^{-20}$
rs10967470	9	2665698	$2.19x10^{-21}$	NA	0.044	$2.19x10^{-21}$	$4.96x10^{-21}$
Model C							
rs6921438	6	44033585	$3.73x10^{-507}$	NA	$3.54x10^{-32}$	$3.73x10^{-507}$	$1.22x10^{-521}$
rs4513773	6	44033504	$4.54x10^{-484}$	$1.13x10^{-143}$	NA	$1.93x10^{-625}$	$3.75x10^{-589}$
rs9472159	6	44027673	$5.87x10^{-454}$	$6.67x10^{-113}$	$3.49x10^{-30}$	$3.65x10^{-563}$	$7.09x10^{-556}$
rs9369434	6	44026385	$4.64x10^{-445}$	$3.27x10^{-65}$	$1.76x10^{-26}$	$8.50x10^{-495}$	$1.18x10^{-504}$
rs1776717	6	44059314	$8.01x10^{-20}$	$5.20x10^{-4}$	$2.77x10^{-5}$	$5.03x10^{-22}$	$4.48x10^{-26}$
rs1776721	6	43998961	$1.05x10^{-19}$	$5.89x10^{-9}$	0.025	$8.84x10^{-27}$	$4.80x10^{-27}$
rs1886979	6	44012879	$2.65x10^{-19}$	$2.12x10^{-6}$	$2.84x10^{-3}$	$3.12x10^{-24}$	$6.44x10^{-26}$
rs9472155	6	44005705	$5.96x10^{-19}$	$1.33x10^{-9}$	0.010	$1.42x10^{-26}$	$2.46x10^{-27}$
rs844294	6	44008685	$1.63x10^{-14}$	$1.95x10^{-5}$	0.041	$1.71x10^{-18}$	$5.41x10^{-19}$
rs4416670	6	44058431	$1.16x10^{-12}$	0.11	$1.49x10^{-4}$	$1.38x10^{-12}$	$1.48x10^{-15}$
rs910611	6	44058829	$3.37x10^{-10}$	$7.04x10^{-6}$	0.076	$1.39x10^{-14}$	$1.07x10^{-14}$
rs6993770	8	106650704	$2.11x10^{-16}$	$6.87x10^{-8}$	$2.67x10^{-3}$	$3.12x10^{-22}$	2.95x10-^{24}
rs16873402	8	106658423	$1.26x10^{-14}$	$1.75x10^{-8}$	0.043	$9.95x10^{-21}$	$2.63x10^{-21}$
rs16873365	8	106627411	$7.36x10^{-12}$	$2.02x10^{-6}$	0.26	$5.26x10^{-16}$	$6.72x10^{-16}$
rs7013321	8	106662734	$3.41x10^{-12}$	NA	$2.21x10^{-3}$	$3.41x10^{-12}$	$2.90x10^{-14}$

rs6993696	8	106650460	$6.23x10^{-12}$	$2.09x10^{-4}$	$2.57x10^{-3}$	$6.00x10^{-15}$	$6.22x10^{-17}$
rs16873291	8	106597206	$4.25x10^{-11}$	$9.77x10^{-7}$	0.031	$1.00x10^{-15}$	$1.04x10^{-16}$
rs1349319	8	106625810	$2.33x10^{-8}$	$1.61x10^{-3}$	$7.71x10^{-3}$	$1.51x10^{-10}$	$4.18x10^{-12}$
rs10738760	9	2681186	$1.91x10^{-34}$	$5.53x10^{-8}$	$9.24x10^{-3}$	$2.94x10^{-40}$	$7.37x10^{-41}$
rs6475920	9	2663933	$3.43x10^{-32}$	$9.54x10^{-8}$	$6.55x10^{-3}$	$2.33x10^{-38}$	$8.21x10^{-39}$
rs4741756	9	2658187	$5.22x10^{-31}$	$2.27x10^{-4}$	0.049	$2.71x10^{-33}$	$1.28x10^{-32}$
rs2375980	9	2682622	$1.15x10^{-27}$	$8.63x10^{-8}$	0.021	$1.01x10^{-33}$	$7.71x10^{-34}$
rs10122587	9	2681951	$4.10x10^{-24}$	NA	0.027	$4.11x10^{-24}$	$2.79x10^{-24}$
rs10967492	9	2671175	$2.39x10^{-21}$	NA	0.19	$2.39x10^{-21}$	$3.15x10^{-20}$
rs10967470	9	2665698	$2.66x10^{-21}$	NA	0.096	$2.66x10^{-21}$	$9.16x10^{-21}$
Model D							
rs6921438	6	44033585	$1.53x10^{-508}$	NA	$1.40x10^{-40}$	$1.53x10^{-508}$	$1.51x10^{-529}$
rs4513773	6	44033504	$2.35x10^{-485}$	$6.36x10^{-136}$	NA	$4.72x10^{-619}$	$1.67x10^{-585}$
rs9472159	6	44027673	$1.61x10^{-454}$	$4.22x10^{-108}$	$1.52x10^{-36}$	$8.63x10^{-559}$	$3.15x10^{-557}$
rs9369434	6	44026385	$6.46x10^{-445}$	$7.63x10^{-63}$	$2.70x10^{-29}$	$3.63x10^{-492}$	$3.43x10^{-501}$
rs1776717	6	44059314	$2.43x10^{-19}$	$2.96x10^{-4}$	$7.08x10^{-6}$	$6.53x10^{-22}$	$1.76x10^{-26}$
rs1776721	6	43998961	$7.12x10^{-20}$	$4.46x10^{-8}$	0.013	$3.12x10^{-26}$	$1.14x10^{-26}$
rs1886979	6	44012879	$1.54x10^{-19}$	$2.15x10^{-6}$	$7.09x10^{-3}$	$1.85x10^{-24}$	$2.03x10^{-25}$
rs9472155	6	44005705	$1.37x10^{-19}$	$4.11x10^{-9}$	$7.43x10^{-3}$	$7.78x10^{-27}$	$1.49x10^{-27}$
rs844294	6	44008685	$2.77x10^{-15}$	$1.48x10^{-5}$	0.048	$2.23x10^{-19}$	$1.65x10^{-19}$
rs4416670	6	44058431	$1.95x10^{-12}$	0.088	$2.05x10^{-4}$	$1.55x10^{-12}$	$1.74x10^{-15}$
rs910611	6	44058829	$3.19x10^{-10}$	$3.45x10^{-6}$	0.11	$7.40x10^{-15}$	$1.70x10^{-14}$
rs6993770	8	106650704	$5.19x10^{-16}$	$5.30x10^{-8}$	0.016	$7.22x10^{-22}$	$9.86x10^{-23}$
rs16873402	8	106658423	$3.72x10^{-14}$	$1.34x10^{-8}$	0.15	$2.91x10^{-20}$	$1.10x10^{-19}$
rs16873365	8	106627411	$2.38x10^{-11}$	$3.87x10^{-6}$	0.36	$2.99x10^{-15}$	$1.14x10^{-14}$
rs7013321	8	106662734	$8.07x10^{-12}$	NA	0.013	$8.07x10^{-12}$	$4.55x10^{-13}$
rs6993696	8	106650460	$1.36x10^{-11}$	$2.41x10^{-4}$	0.040	$1.52x10^{-14}$	$3.72x10^{-15}$
rs16873291	8	106597206	$9.39x10^{-11}$	$1.37x10^{-6}$	0.071	$3.23x10^{-15}$	$1.30x10^{-15}$
rs1349319	8	106625810	$3.87x10^{-8}$	$2.46x10^{-3}$	0.057	$3.66x10^{-10}$	$7.71x10^{-11}$
rs10738760	9	2681186	$1.36x10^{-35}$	$1.06x10^{-8}$	0.046	$3.32x10^{-42}$	$1.35x10^{-40}$
rs6475920	9	2663933	$9.18x10^{-33}$	$2.67x10^{-8}$	0.018	$1.68x10^{-39}$	$1.36x10^{-38}$
rs4741756	9	2658187	$9.04x10^{-32}$	$1.28x10^{-4}$	0.071	$2.35x10^{-34}$	$9.51x10^{-33}$
rs2375980	9	2682622	$3.07x10^{-28}$	$3.55x10^{-8}$	0.015	$1.02x10^{-34}$	$1.70x10^{-34}$
rs10122587	9	2681951	$5.73x10^{-25}$	NA	0.020	$5.73x10^{-25}$	$6.91x10^{-25}$
rs10967492	9	2671175	$3.72x10^{-22}$	NA	0.091	$3.72x10^{-22}$	$3.50x10^{-21}$
rs10967470	9	2665698	$5.48x10^{-22}$	NA	0.045	$5.48x10^{-22}$	$1.30x10^{-21}$
Model E							
rs6921438	6	44033585	$6.58x10^{-506}$	NA	$2.91x10^{-39}$	$6.58x10^{-506}$	$7.66x10^{-524}$
rs4513773	6	44033504	$6.46x10^{-482}$	$3.04x10^{-138}$	NA	$6.76x10^{-618}$	$1.66x10^{-583}$
rs9472159	6	44027673	$9.84x10^{-452}$	$4.86x10^{-110}$	$4.30x10^{-35}$	$4.42x10^{-558}$	$1.30x10^{-552}$
rs9369434	6	44026385	$2.44x10^{-442}$	$3.05x10^{-65}$	$5.33x10^{-28}$	$1.10x10^{-492}$	$6.10x10^{-498}$
rs1776717	6	44059314	$9.92x10^{-20}$	$2.53x10^{-4}$	$1.00x10^{-5}$	$2.40x10^{-22}$	$8.47x10^{-27}$
rs1776721	6	43998961	$5.41x10^{-20}$	$2.57x10^{-8}$	0.020	$1.47x10^{-26}$	$1.30x10^{-26}$
rs1886979	6	44012879	$2.55x10^{-19}$	$1.66x10^{-6}$	0.013	$2.41x10^{-24}$	$7.07x10^{-25}$
rs9472155	6	44005705	$1.74x10^{-19}$	$3.04x10^{-9}$	0.015	$7.84x10^{-27}$	$4.93x10^{-27}$
rs844294	6	44008685	$8.15x10^{-15}$	$1.39x10^{-5}$	0.086	$6.33x10^{-19}$	$1.20x10^{-18}$
rs4416670	6	44058431	$1.86x10^{-12}$	0.11	$2.89x10^{-4}$	$2.16x10^{-12}$	$3.14x10^{-15}$

rs910611	6	44058829	$2.35x10^{-10}$	$1.99x10^{-6}$	0.11	$3.34x10^{-15}$	$8.62x10^{-15}$
rs6993770	8	106650704	$1.34x10^{-16}$	$3.63x10^{-8}$	0.018	$1.27x10^{-22}$	$2.52x10^{-23}$
rs16873402	8	106658423	$1.24x10^{-14}$	$8.58x10^{-9}$	0.16	$6.38x10^{-21}$	$3.39x10^{-20}$
rs16873365	8	106627411	$8.10x10^{-12}$	$4.01x10^{-6}$	0.37	$9.32x10^{-16}$	$5.17x10^{-15}$
rs7013321	8	106662734	$3.64x10^{-12}$	NA	0.014	$3.64x10^{-12}$	$2.48x10^{-13}$
rs6993696	8	106650460	$5.82x10^{-12}$	$1.73x10^{-4}$	0.047	$4.78x10^{-15}$	$1.67x10^{-15}$
rs16873291	8	106597206	$3.14x10^{-11}$	$7.92x10^{-7}$	0.066	$6.56x10^{-16}$	$2.99x10^{-16}$
rs1349319	8	106625810	$3.53x10^{-8}$	$1.84x10^{-3}$	0.046	$2.60x10^{-10}$	$4.55x10^{-11}$
rs10738760	9	2681186	$6.77x10^{-35}$	$3.90x10^{-9}$	0.034	$4.65x10^{-42}$	$1.61x10^{-40}$
rs6475920	9	2663933	$1.85x10^{-32}$	$9.61x10^{-9}$	0.022	$1.20x10^{-39}$	$1.94x10^{-38}$
rs4741756	9	2658187	$1.32x10^{-31}$	$6.02x10^{-5}$	0.092	$1.26x10^{-34}$	$1.22x10^{-32}$
rs2375980	9	2682622	$7.94x10^{-28}$	$1.45x10^{-8}$	0.022	$9.69x10^{-35}$	$4.67x10^{-34}$
rs10122587	9	2681951	$1.47x10^{-24}$	NA	0.024	$1.47x10^{-24}$	$2.56x10^{-24}$
rs10967492	9	2671175	$6.75x10^{-22}$	NA	0.10	$6.76x10^{-22}$	$9.22x10^{-21}$
rs10967470	9	2665698	$9.41x10^{-22}$	NA	0.045	$9.41x10^{-22}$	$2.39x10^{-21}$

All analyses were adjusted for age, sex, and principal components. Model B was additionally adjusted for hypertension; model C for smoking; model D for central obesity; model E for the presence of a metabolic syndrome; [*]inverse variance meta-analysis; [†]effective sample size weighted meta-analysis

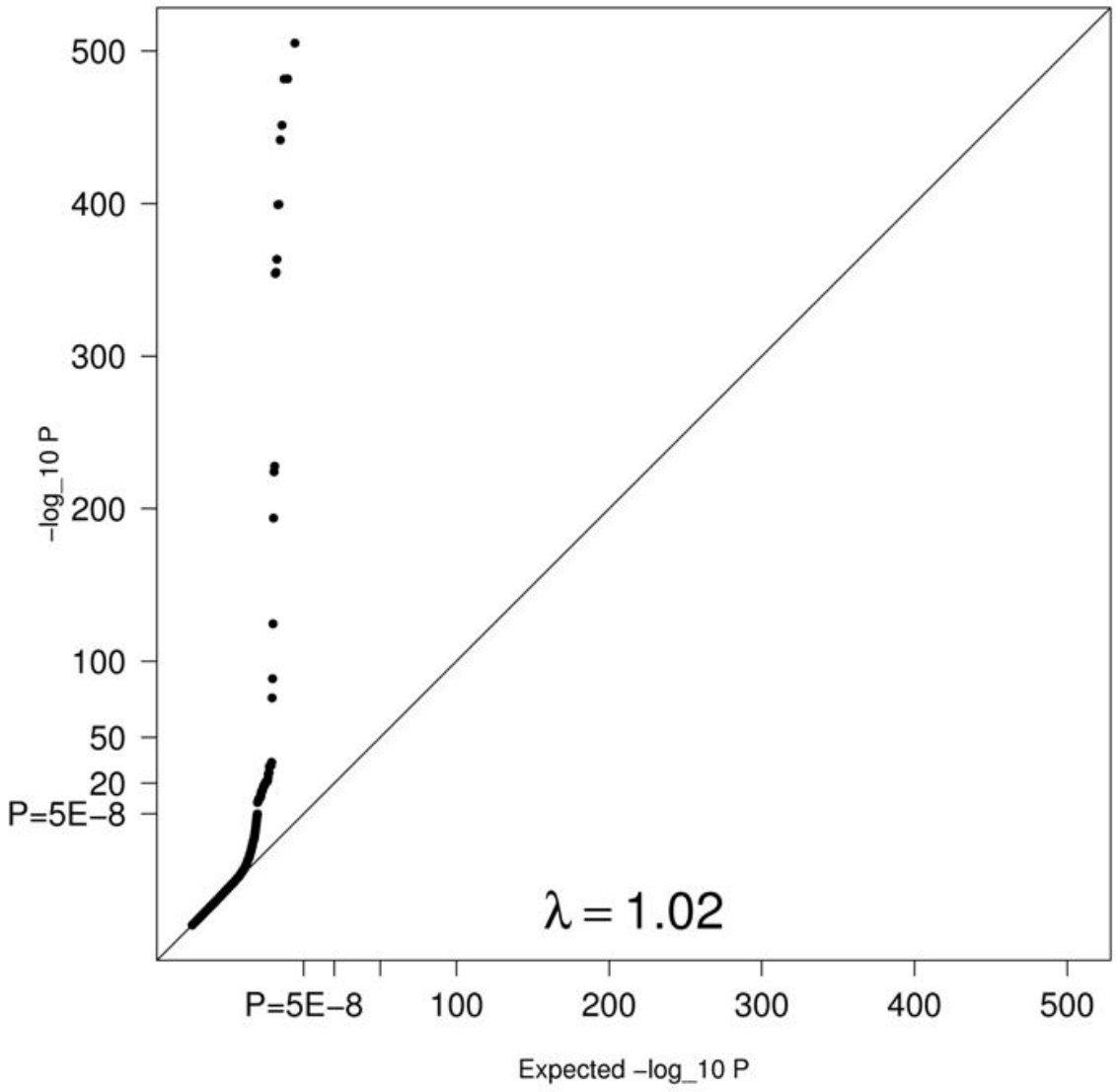
Online Table V: Candidate gene association studies of VEGF variants and circulating VEGF levels

Author	Population	N	Sample	SNP					
				rs699947	rs1570360*	rs833061	rs2010963*	rs3025039*	-2549 18bp I/D
Steffensen [22]	ovarian cancer patients	143	serum	C ↑ VEGF	NS	T ↑ VEGF	C ↑ VEGF	NS	-
Costa [16]	post-menopausal women	252	serum	-	-	-	NS	NS	-
Medford [17]	patients with (and at risk of) ARDS	70	plasma	-	-	-	-	NS	-
Kamoun [23]	Behcet patients and healthy controls	135	serum	-	-	-	NS	NS	D ↑ VEGF
Langsenlehner [18]	healthy persons	81	plasma	NS	-	NS	NS	NS	-
Petrovic [29]	PDR patients	104	serum	-	-	-	CC ↑ VEGF	-	-
Mateo [19]	AD patients and healthy controls	117	serum	NS	-	-	NS	-	-
Balasubramanian, [20]	post-menopausal women	62	serum and plasma	-	-	NS	NS	NS	-
Zhai [24]	ARDS patients	71	plasma	-	-	NS	NS	T ↓ VEGF	-
Berrahmoune [21]	community sample	647	plasma	-	-	NS	NS	NS	-
Ferrante [25]	IBD patients and healthy controls	1,142	serum	NS	GG ↑ VEGF	-	NS	NS	-
Krippl [26]	post-menopausal women	21	plasma	-	-	-	-	T ↓ VEGF	-
Awata [27]	healthy persons	64	serum	-	NS	NS	CC ↑ VEGF	NS	-
Renner [28]	healthy persons	23	plasma	-	-	-	-	T ↓ VEGF	-
Present study	**community sample**	**3,527**	**serum**	**C ↑ VEGF $p=2.33x10^{-7}$ (beta±SE= 0.13±0.02)**	**NA**	**T ↑ VEGF $p=3.05x10^{-7}$ (beta±SE= 0.12±0.02)**	**NA**	**NA**	**NA**

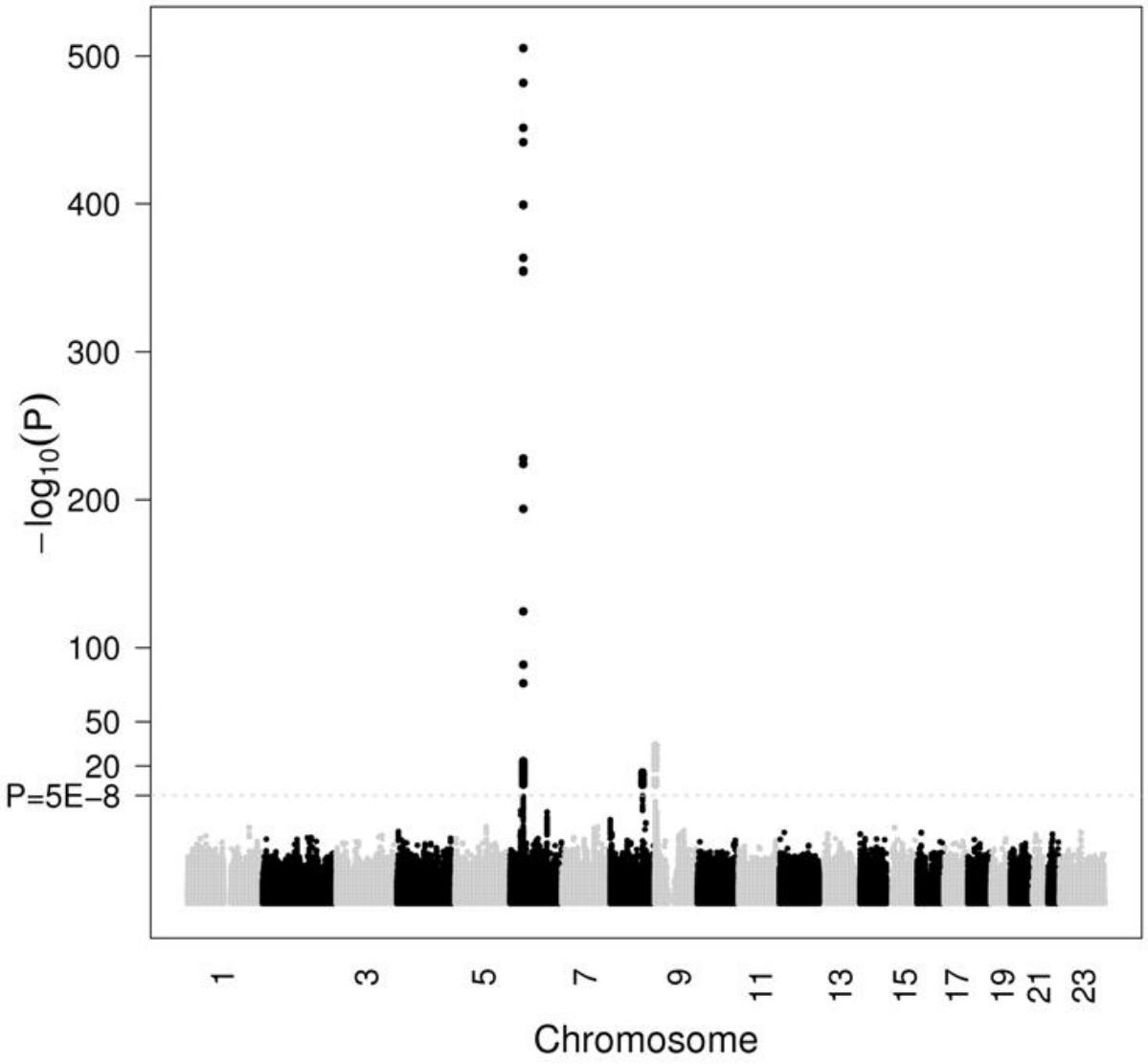
AD: Alzheimer Disease; ARDS: Acute Respiratory Distress Syndrome; IBD: Inflammatory Bowel Disease; HAM/TSP: HTLV-I- associated myelopathy/tropical spastic paraparesis; NS: non significant (no association); NA: not available; PDR = Proliferative Diabetic Retinopathy;
*these SNPs were not available in our GWAS and were nota in linkage disequilibrium with the VEGF SNPs yielding a genome-wide significant association with VEGF levels ($r^2 < 0.095$)

C. Supplemental Figures

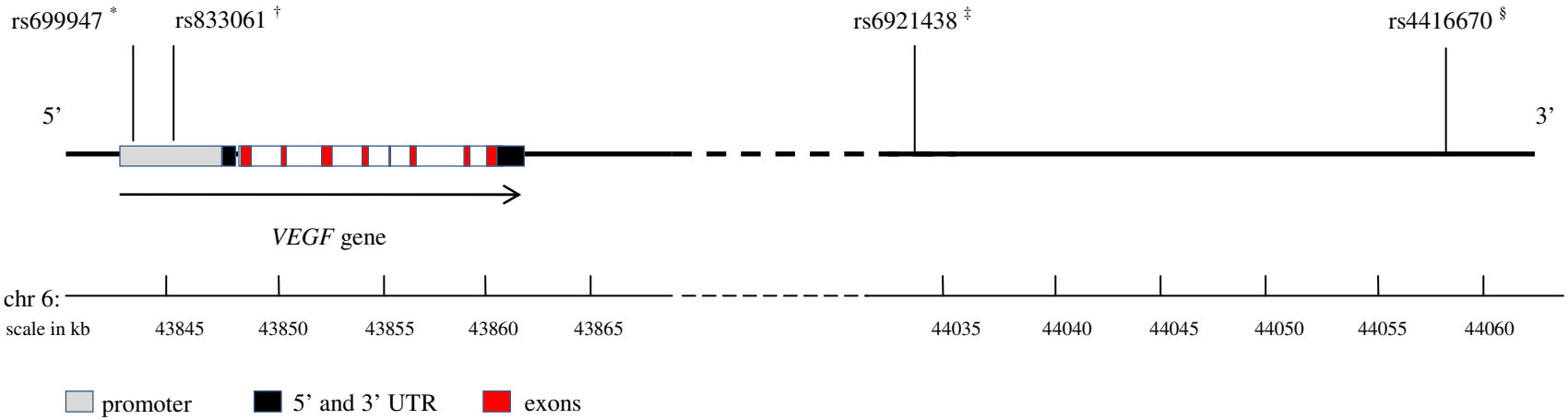
Online Figure I: Quantile-quantile QQ-plot showing the observed versus the expected p-values after meta-analysis for serum VEGF levels (the solid line shows the distribution under the null-hypothesis)



Online Figure II: Manhattan plot showing individual p-values against their genomic position for GWAS of serum VEGF levels. Within each chromosome (x-axis), results are plotted left to right from p-terminal end. Dashed line indicates preset threshold for genome-wide significance, p=5.0x10-8; solid line threshold for suggestive associations, $p=4.0x10^{-7}$



Online Figure III: Genomic organization of the VEGF gene and localization of the SNPs identified in the genetic association and transcriptomic studies



*chr6:43844367, p=2.33x10^{-7} replicates findings from previous association studies (Supplementary Table IV)
†chr6:43845464, p=3.05x10^{-7}, replicates findings from previous association studies (Supplementary Table IV)
‡ chr6:44033585, p=6.11x10^{-506}, top SNP, explains 41.2% of the phenotypic variance in the FHS
§ chr6:44058431, p=1.47x10^{-12}, associated with serum VEGF levels independently of rs6921438 in conditional GWAS, explains 1.5% of the phenotypic variance in the FHS

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