Clinical and Genetic Correlates of Circulating Angiopoietin-2 and Soluble Tie-2 in the Community

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Background—Experimental studies suggest that endothelial growth factors play an important role in angiogenesis and vascular remodeling. The clinical and genetic correlates of circulating angiopoietin-2 (Ang-2) and its soluble receptor/regulator Tie-2 (sTie-2) have not been determined in a community-based sample.

Methods and Results—Serum Ang-2 and sTie-2 were assayed in 3778 third-generation cohort participants of the Framingham Heart Study (mean age, 40±9 years; 53% women). Clinical correlates and heritability of both biomarkers were assessed using generalized estimating equations and variance-component analyses. Ang-2 levels were higher and sTie-2 levels were lower in women than in men. Ang-2 was positively related to age, smoking, systolic blood pressure, hypertension treatment, and diabetes (P<0.05 for all) but was inversely associated with total cholesterol and diastolic blood pressure (P<0.0001 for both), and sTie-2 was positively associated with body mass index, diabetes, and triglycerides but was inversely related to age, alcohol consumption, and glomerular filtration rate (P<0.05 for all). Both Ang-2 and sTie-2 were higher in participants with metabolic syndrome (P<0.005), with stronger associations of Ang-2 with blood pressure traits and of sTie-2 with obesity-dyslipidemia components. Heritability estimates for Ang-2 and sTie-2 were 27% and 56%, respectively (P<0.0001). A region on chromosome 9 was significantly linked to circulating sTie-2 levels (logarithm of the odds score, 8.31).

Conclusion—Circulating levels of Ang-2 and sTie-2 are heritable traits associated with cardiovascular disease risk factors, including the metabolic syndrome. These observations are consistent with the notion that angiogenesis and vascular remodeling are determined in part by genetic influences and associated with metabolic risk factors. (Circ Cardiovasc Genet. 2010;3:300-306.)

Key Words: angiopoietins ■ vascular remodeling ■ heritability ■ metabolic syndrome ■ CVD risk factors ■ epidemiology ■ growth substances

Vascular remodeling, a process characterized by progressive structural and functional changes in the vessel wall, is an adaptive response of the vessel to hemodynamic and biochemical stressors and a precursor of overt cardiovascular disease (CVD). Understanding the biological basis of vascular remodeling, therefore, is critical for preventing CVD. The endothelium plays a central role in vascular remodeling through, in part, endothelium-derived signaling proteins and receptors that mediate intercellular communication.1,2 Angiopoietins are an important group of endothelial growth factors that modulate angiogenesis3 and vascular remodeling.4 Although the various angiopoietins bind to the same endothelial tyrosine kinase receptor Tie-2, they seem to have distinct, context-dependent biological functions. Binding of angiopoietin-1 (Ang-1) to Tie-2 leads to prolonged endothelial cell survival, maintains the endothelium in a quiescent state,5 and supports the maturation of new vessels.6 Angiopoietin-2 (Ang-2), on the other hand, functions as a competitor inhibitor of Ang-1 for Tie-2 binding,2 thereby inhibiting Ang-1/Tie-2 signaling.7 Preformed Ang-2 in the Weibel-Palade bodies in endothelial cells is readily available for rapid release and primes the endothelium to respond to stressors. Ang-2 also promotes vascular endothelial growth factor (VEGF)-induced neovascularization. Recently, a solu-
Correlates of Angiopoietin-2 and Soluble Tie-2

Clinical Correlates

In smaller clinical studies, altered circulating concentrations of Ang-2, Tie-2, or both were observed in patients with peripheral artery disease, acute coronary syndrome, and heart failure and reported to predict incident myocardial infarction. Furthermore, the levels of these biomarkers were associated with measures of subclinical disease and target organ damage in hypertension. Although these preliminary data suggest an important role for angiopoietins along the CVD continuum, the clinical and genetic correlates of Ang-2 and sTie-2 have not been evaluated in a large community-based sample. We hypothesize that circulating Ang-2 and Tie-2 are associated with traditional CVD and metabolic risk factors and with target organ damage (ECG-left ventricular hypertrophy [LVH]), and they are heritable traits influenced by select genetic loci.

Methods

In 2002, the third-generation cohort of the Framingham Heart Study was initiated, and a total of 4095 participants with at least 1 parent in the offspring cohort were included. The data for these analyses were obtained at the first examination cycle. Participants were comprehensively evaluated, including anthropometric measurements, biochemical assessment for traditional CVD risk factors, and a medical history and physical examination by a study physician. These analyses included only third-generation cohort participants. Overall, 317 participants were excluded for the following reasons: prevalent CVD (n=66), serum creatinine >2 mg/dL (n=1), missing data on Ang-2 or Tie-2 (n=126), and missing covariates (n=124). After exclusions, 3778 participants remained eligible for the present analyses. Written informed consent was provided by all participants, and the Institutional Review Board at the Boston University Medical Centre (Boston, Mass.) approved the study protocol.

Biomarker Measurement

Blood was drawn in the early morning after a 12-hour fast. Blood samples were immediately centrifuged and stored at −80°C until biomarkers were assayed. Circulating concentrations of free Ang-2 and sTie-2 in serum were assayed using commercial assays (R&D Inc). The average interassay coefficients of variation were 5.7% for Ang-2 and 3.2% for sTie-2. VEGF A, its soluble (R&D Inc). The average interassay coefficients of variation were

Electrocardiography

Presence of LVH was determined based on a standard 12-lead ECG when at least 1 of the following criteria were fulfilled: precordial RV5, RV6+SV1, or SV2 >3.5 mV; R wave in AVL >11 mV; R wave in left precordial leads ≥2.5 mV; S wave in right precordial leads ≥2.5 mV; and R wave in lead I+S wave in lead III ≥2.5 mV.

Statistical Analyses

Clinical Correlates

Ang-2 and sTie-2 were natural-logarithmically transformed to normalize their skewed distributions. Significant correlates of Ang-2 and sTie-2 (each biomarker considered separately) were identified using forward stepwise selection procedures (P≤0.1 for model entry) from a set of candidate variables (age, sex, systolic and diastolic blood pressure [BP], antihypertensive medication, diabetes, total cholesterol, high-density lipoprotein [HDL] cholesterol, triglycerides, smoking, body mass index [BMI], alcohol consumption, and estimated glomerular filtration rate [eGFR]). Then, we used generalized estimating equations through the Compound Symmetry Correlation Matrix to account for familial correlation within our sample.

In secondary analyses, we replaced each significant continuous predictor variable (except for age) with a categorical counterpart frequently used in clinical settings: hypertension (BP ≥140/90 mm Hg or antihypertensive medication); obesity (BMI ≥30 kg/m²); abdominal obesity (waist circumference ≥102 cm in men or ≥89 cm in women); low HDL cholesterol (<40 mg/dL in men or <50 mg/dL in women); high triglycerides (≥150 mg/dL or use of lipid-lowering medication); and the metabolic syndrome (defined as the presence of ≥3 of the following features: increased waist circumference [mentioned earlier], elevated BP [≥130/85 mm Hg or antihypertensive treatment], hyperglycemia [fasting glucose ≥100 mg/dL or treatment for elevated glucose], hypertriglyceridemia [≥150 mg/dL or lipid-lowering treatment], and low HDL cholesterol [mentioned earlier]). We evaluated the pair-wise correlations of Ang-2 and sTie-2 with other vascular growth factors measured in this cohort (ie, VEGF, sFlt-1, HGF) using Pearson correlation coefficients, adjusting for age and sex.

Association of Biomarker Concentrations With ECG-LVH

We related Ang-2 and sTie-2 concentrations to LVH using logistic regression models, adjusting for age and sex. The multivariable models also were adjusted for all covariates that were significantly related to the respective biomarker (Ang-2 or sTie-2) in the previous analyses (clinical correlates).

Genetic Correlates: Heritability and Linkage Analyses

Variance-component models as implemented in the software package SOLAR were used to estimate heritability for log-biomarker levels using 2 models: model 1 adjusted for age and sex, and model 2 further adjusted for all significant clinical correlates of the respective biomarkers that were identified in the previous analyses detailed earlier. Data from 3768 (10 participants of our sample lacked genotypic data) individuals belonging to 1796 core families (online-only Data Supplement Table I), which constitute 805 pedigrees, were used for a multipoint linkage analysis for Ang-2 and Tie-2 with the SOLAR software. The number of core families was larger than the number of pedigrees because members of some core families were second-degree relatives and, therefore, represent the same pedigree. Adjustment was performed for age and sex only (model 1) and for those covariates that were significantly associated with Ang-2 and sTie-2 in our previous analyses (model 2). The genetic data set included 687 microsatellite markers. To quantify the evidence for linkage, the logarithm (base 10) of the likelihood ratio for linkage was calculated.

Results

The clinical and biochemical characteristics of our sample are provided in Table 1. Our sample comprised young to middle-aged individuals and included slightly more women than men. Information about the number of siblings per family is provided in supplemental Table I. Circulating concentrations of Ang-2 and sTie-2 were not correlated (r=0.004; P=0.8) in age- and sex-adjusted models. Pair-wise age- and sex-adjusted correlations among Ang-2, sTie-2, VEGF, sFlt-1, and HGF are provided in supplemental Table II. Overall, correlations between endothelial biomarker concentrations were rather low; modest associations were observed between Ang-2 and HGF and sFlt-1, and between sTie-2 and VEGF and HGF.
Clinical Correlates of Ang-2 and sTie-2

Ang-2 levels were higher in women than in men and were positively associated with age, smoking, antihypertensive medication, systolic BP, and diabetes (Table 2). Inverse associations were observed with diastolic BP and total cholesterol (Table 2). The multivariable model explained 6.8% of the interindividual variation in circulating Ang-2 concentrations. We observed a positive association with hypertension when we replaced systolic and diastolic BP with the binary variable ($\beta=0.054$; 95% CI, 0.016 to 0.092; $P=0.005$).

Noting the divergent directions of the associations with systolic and diastolic BP, we substituted the 2 measures with pulse pressure and observed a positive correlation between Ang-2 and pulse pressure ($\beta=0.0221$; 95% CI, 0.0081 to 0.0361 per 1 SD-increment; $P=0.002$). Replacing all significant continuous traits that are part of the definition of the metabolic syndrome, Ang-2 also was positively associated with the metabolic syndrome as a binary trait ($\beta=0.048$; 95% CI, 0.015 to 0.082; $P=0.0047$). Ang-2 levels in our sample were related to ECG-determined LVH (prevalence, 8.1%; odds ratio, 1.02; 95% CI, 1.01 to 1.04; $P=0.011$ per 1-unit increment in log-Ang-2 levels [a 2.72-($e^{1}$)-fold increment in Ang-2 in natural units]).

Circulating sTie-2 concentrations were higher in men than in women and were positively related to BMI, diabetes, and triglycerides. Inverse associations with age, eGFR, and alcohol consumption were noted. The multivariable model explained 3.8% of the interindividual variation of circulating sTie-2 concentrations. Replacing BMI in the multivariable model with the following traits revealed positive associations: obesity ($\beta=0.046$; 95% CI, 0.025 to 0.068; $P<0.0001$, indicating $\approx5\%\ [e^{0.046}]$ higher Tie-2 levels in participants with obesity than in participants without obesity), waist circumference ($\beta=0.016$; 95% CI, 0.005 to 0.026; $P=0.004$), abdominal obesity ($\beta=0.029$; 95% CI, 0.01 to 0.049; $P=0.003$), and the metabolic syndrome ($\beta=0.047$; 95% CI, 0.025 to 0.068; $P<0.0001$). Concentrations of sTie-2 were not significantly associated with ECG-determined LVH (odds ratio, 1.00; 95% CI, 0.097 to 1.03; $P=0.95$).

Genetic Correlates of Ang-2 and sTie-2

Heritability estimates were moderate for Ang-2 (27%) and substantial for sTie-2 (56%) in age-, sex-, and multivariable-adjusted models (Table 3). Multipoint log of the odds (LOD) scores from the genome-wide linkage analysis for Ang-2 and sTie-2 levels are shown in supplemental Figures I and II. After multivariable adjustment, the highest LOD score for Ang-2 was observed on chromosome 5 (marker D5S2006) in the butyrophilin-like 8 gene (LOD score, 2.41), which is below the acceptable threshold for significant linkage (LOD score, 3). For sTie-2, the highest linkage peak (LOD score, 8.31) was identified at 60 cm on chromosome 9, with genotyped markers GATA5E06 at 58.26 cm and GATA7E08 at 58.27 cm nearby. The wide highest linkage region (LOD score, >3) extends from 40 to 72 cm on chromosome 9 and includes the Tie-2 gene as well as a number of genes with known or putative functions in cardiovascular physiology (see Discussion). The second linkage peak (LOD score, 2.91) was identified at 158 cm on chromosome 12, with the genotyped marker ATA29A06 at 160.68 cm nearby, which is close to the TMEM132D (transmembrane protein 132D) gene. However, the peak LOD score (2.91) was slightly below the accepted threshold for significant linkage.

Discussion

We evaluated clinical and genetic correlates of Ang-2 and the soluble form of its receptor (sTie-2) in a large community-based sample including $\approx4000$ individuals. A sexual dimorphism for both biomarker concentrations was observed, with Ang-2 levels
being significantly higher and sTie-2 concentrations significantly lower in women than in men. Relations to age also were opposite in directionality for the 2 markers, with Ang-2 increasing with age and sTie-2 declining with age.

Both Ang-2 and sTie-2 were positively related to diabetes and to the presence of the metabolic syndrome, but the 2 biomarkers were associated with different components of metabolic syndrome. Ang-2 was more strongly associated with BP and pulse pressure, whereas sTie-2 was associated with obesity and hypertriglyceridemia, components of the atherogenic triad.

Circulating Ang-2 and sTie-2 concentrations demonstrated moderate (27%) to substantial (56%) heritability, respectively.

Finally, we obtained strong evidence for linkage of a segment on chromosome 9 with sTie-2 levels (LOD score, 8.31).

### Sexual Dimorphism in Circulating Ang-2 and Tie-2 Concentrations

Gender differences in vascular function have been well described, and differences in endothelium-derived vascular growth factors between men and women might be a contributing factor. Sexual dimorphism has been reported for several endothelium-derived growth factors. Experimental and animal studies support the notion that sex hormones influence vascular growth factor expression. For example, estrogen/estradiol has been shown to modulate expression of Ang-1 and VEGF. Recent evidence suggests that sTie-2 and Ang-2 levels also are modulated by estrogen. In agreement with our observation of higher Ang-2 levels in women, estrogen induces Ang-2 expression in several tissues.

Our findings regarding sex-related differences in distributions of Ang-2 and sTie-2 deserve additional confirmation, and the underlying mechanisms should be elucidated.

### Clinical Correlates of Ang-2

Circulating Ang-2 concentrations showed highly significant positive associations with major vascular risk factors, in particular BP and pulse pressure, hypertension, smoking, and diabetes. The positive association with diabetes is in agreement with 2 previous clinical studies. Increased levels of...
Ang-2 have been implicated in the development of diabetic microvascular complications, including retinopathy. Ang-2 and VEGF play an important role in the initiation of neovascularization and stabilization of incipient vascular tubes. Ang-2 may have a role in facilitating VEGF-initiated neovascular sprouting by impairing Ang-1 stabilization and maintenance of existing tubes. The positive association of Ang-2 levels with BP traits agrees with several previous studies reporting that in patients with hypertension, Ang-2 levels were elevated, correlated with target organ damage, and predicted the incidence of myocardial infarction. Given that Ang-2 and Ang-1 are competing for the same endothelial-bound receptor and that Ang-2 thereby inhibits Ang-1/Tie-2 signaling-related vessel stabilization, it is possible that high levels of Ang-2 serve as a surrogate marker for reduced Ang-1/Tie-2 signaling.

Several potential explanations exist for the positive relationships of Ang-2 with BP. First, dysregulation of vascular endothelial growth factors, including increased Ang-2 levels, independent of CVD risk factors, might lead to a paucity of mature small vessels called microvascular rarefaction, which is a common finding in patients with hypertension. Microvascular rarefaction increases the transmission of pulsatile load to the microcirculation, which increases the risk of target organ damage. Second, experimental data suggest that Ang-1 favorably influences vascular remodeling through antiinflammatory and antiatherogenic properties and that cartilage oligomeric matrix protein-Ang-1, a derivative of Ang-1, prevents the development of hypertension and associated target organ damage in prehypertensive spontaneously hypertensive rats. Therefore, reduced Ang-1/Tie-2 activity could be causally related to the development of elevated BP. In this context, Ang-2 also has been reported to be an autocrine regulator of endothelial cell responses to inflammatory stimuli, with a permissive role for the effects of proinflammatory cytokines. A third possible explanation is that circulating Ang-2 levels are increased in response to BP-induced vascular damage. Elevated BP and smoking adversely affect vascular remodeling, so increased circulating levels of Ang-2 might be an adaptive response to the increasing burden of CVD risk factors. The positive association of circulating Ang-2 with smoking is consistent with experimental studies that demonstrate a pattern of heightened Ang-2 gene expression in endothelial cells of smokers (compared with nonsmokers), consistent with heightened stress-induced senescence of endothelial cells in smokers.

Our finding of an association between Ang-2 and ECG-determined LVH not only is consistent with angiogenesis being necessary for cardiac hypertrophy, but also is in good agreement with recent basic science data suggesting an antihypertrophic effect of Ang-1 on cardiac myocytes through binding to integrin receptors. As detailed earlier, increased soluble Ang-2 levels could serve as a surrogate marker for reduced Ang-1 activity, which would explain the positive association between Ang-2 levels and LVH.

**Clinical Correlates of sTie-2**

Concentrations of sTie-2 showed highly significant associations with the metabolic syndrome and its components, including diabetes and triglycerides, and indices of body composition, including BMI, waist circumference, obesity, and abdominal obesity. These findings are consistent with the results of a small clinical study and agree well with the positive association of other endothelial growth factors, including HGF, with obesity and the metabolic syndrome. Given the strong interdependence of adipose tissue and angiogenesis, this positive association between sTie-2 (the key endothelial receptor for all angiopoietins) and the metabolic syndrome and obesity (including abdominal obesity) is not surprising. Adipose tissue is well vascularized, and its growth requires the presence of angiogenic factors and their receptor. It has been shown in mouse models of obesity that Ang-2 expression was increased in subcutaneous adipose tissue and that inhibitors of angiogenesis effectively reduced weight and adipose tissue mass. On the other hand, the adipose tissue itself might be a source of angiopoietins and their receptor because adipose tissue represents a very active endocrine organ that produces a broad array of hormones, including promoters and inhibitors of angiogenesis. It is not clear what the presence of sTie-2 indicates in this context. Although sTie-2 is elaborated by adipose-related cell types in response to upstream signals and promotes increased adipogenesis, sTie-2 also inhibits angiogenesis by limiting circulating Ang-1 and Ang-2 from presentation to endothelial Tie-2, with consequent loss of vessel stabilization signaling. Thus, it is unclear whether the positive association of sTie-2 with BMI is due to increased elaboration of the growth factor by the expanded fat compartment or whether it is in response to the greater need for angiogenesis as the compartment expands. Tie-2 is expressed in glomerular capillary endothelial cells. In this light, our finding of an inverse association of sTie-2 with eGFR has intriguing implications on whether impaired angiogenesis could contribute to impaired renal function, as has been described for certain nephropathies. Alternatively, lower eGFR may be associated with greater Tie-2 expression. The association with alcohol intake is consistent with experimental observations that ethanol increases angiogenesis through a Tie-2-dependent pathway.

**Genetic Correlates of Ang-2 and sTie-2**

We observed that 56% of the variation in circulating sTie-2 concentration was due to genetic factors. Ang-2 levels had a moderate heritability of 27%. To our knowledge, this study is the first to report heritability estimates for circulating Ang-2 and sTie-2 concentrations. These heritability estimates are in excellent agreement with previous reports about substantial heritability of circulating levels of other endothelial-derived growth factors and support the notion that circulating levels of angiogenic peptides partly are genetically determined. This concept is supported further by experimental studies reporting that expression of Ang-1, sTie-2, and other angiogenic biomarkers in response to chronic hypoxia differ among mouse strains of varied genetic backgrounds.

Multipoint linkage analyses revealed strong evidence for linkage (LOD score, 8.31) on chromosome 9 with circulating sTie-2 concentrations. The linkage peak is relatively broad and includes the Tie-2 gene, which is the most plausible explanation for this linkage peak. In addition, several other
interesting candidate genes are in the linked region, including the DDX58 (DEAD box polypeptide 58) gene, also known as retinoid acid inducible gene 1, which is expressed in macrophages and has been suggested to play a role in the development of atherosclerotic lesions. Furthermore, the NRP2 gene (encoding atrial natriuretic peptide receptor B precursor) is located in the critical linkage region. Another interesting gene is the DNAJB5 gene, belonging to the DnaJ/Hsp40 (heat shock protein 40) family, which recently has been implicated in cardiac hypertrophy.

Strengths and Limitations

The large community-based design, the familial structure of our study (facilitating heritability and linkage analyses), and the availability of a broad array of carefully assessed covariates strengthen our analyses. However, we acknowledge several limitations. Both biomarkers were measured only once in each individual, which likely led to an underestimation of the strength of the associations between biomarker concentrations and independent variables. The genomic coverage of linkage analyses is not as good as for genome-wide association analyses, and therefore, we might have missed some genetic loci that might be associated with the biomarker concentrations but were not in strong linkage disequilibrium with 1 of the 687 genetic markers we used. The cross-sectional design precludes any causal inferences. Our sample is of young to middle-aged individuals of European descent. The generalizability of our results to other age groups or ethnicities is unknown. Our findings require replication in independent samples, and additional research is warranted to determine whether the observed associations are clinically meaningful.

Conclusions

Circulating sTie-2 and Ang-2 concentration levels were correlated with key CVD and metabolic risk factors consistent with the notion that angiopoietins are involved in vascular remodeling in response to increased CVD risk factor burden. We also found that a moderate-to-substantial proportion of the variation in circulating levels of these proteins is heritable. Our results require confirmation in future studies.

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Disclosures

Dr Mitchell is owner of Cardiovascular Engineering Inc, a company that designs and manufactures devices that measure vascular stiffness.

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**CLINICAL PERSPECTIVE**

Experimental studies suggest that endothelial growth factors play an important role in angiogenesis and vascular remodeling. We evaluated the clinical and genetic correlates of circulating angiopoietin-2 (Ang-2) and its soluble receptor/regulator Tie-2 (sTie-2) in a community-based sample. Ang-2 levels were higher and sTie-2 levels were lower in women than in men. Ang-2 was positively related to age, smoking, systolic blood pressure, hypertension treatment, and diabetes, whereas sTie-2 was positively associated with body mass index, diabetes, and triglycerides. Circulating Ang-2 and sTie-2 were higher in participants with the metabolic syndrome, with stronger associations of Ang-2 with blood pressure traits and of sTie-2 with obesity-dyslipidemia components. Heritability estimates for Ang-2 and sTie-2 were 27% and 56%, respectively. A region on chromosome 9 was significantly linked to circulating sTie-2 levels (log of the odds score, 8.31). These observations are consistent with the concept that angiogenesis and vascular remodeling are influenced by genetic factors and associated with metabolic risk factors.
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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Number of siblings per family in the study sample

Supplemental Table 2. Correlation matrix for natural-logarithmically transformed circulating concentrations of angiopoietin-2 (Ang-2), its soluble receptor sTie-2, vascular endothelial growth factor A (VEGF), its receptor sFlt-1 and hepatocyte growth factor (HGF)

Supplemental Figure 1. Multipoint Logarithm of the odds (LOD) score (y-axis) from the genome wide linkage analysis for Ang-2. X-axis: Chromosomal position. Analyses were adjusted for all significant correlates of Ang-2 (Table 2).

Supplemental Figure 2. Multipoint Logarithm of the odds (LOD) score (y-axis) from the genome wide linkage analysis for sTie-2 levels. X-axis: Chromosomal position. Analyses were adjusted for all significant correlates of sTie-2 (Table 2).
**Supplemental Table 1.** Number of siblings per family in the study sample

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“0” siblings indicates families with 1 Third Generation participant
**Supplemental Table 2.** Correlation matrix for natural-logarithmically transformed circulating concentrations of angiopoietin-2 (Ang-2), its soluble receptor sTie-2, vascular endothelial growth factor A (VEGF), its receptor sFlt-1 and hepatocyte growth factor (HGF)

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<th>Log-VEGF</th>
<th>Log-HGF</th>
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<td>-0.0451*</td>
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<td>0.05893*</td>
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**p<0.0001
*p<0.01**
In_Ang2: multivariable adjusted

Chromosome

Multipoint LOD score

0.0 0.5 1.0 1.5 2.0 2.5 3.0
In_Tie2: multivariable adjusted

Multipoint LOD score

Chromosome