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Clinical and genetic correlates of Circulating Angiopoetin-2 and soluble Tie-2 in the Community

Wolfgang Lieb, MD*, **Justin Zachariah, MD***, **Vanessa Xanthakis, MS**, **Radwan Safa, MS**, **Ming-Huei Chen, PhD**, **Lisa M. Sullivan, PhD**, **Martin G. Larson, ScD**, **Holly M. Smith, MS**, **Qiong Yang, PhD**, **Gary F. Mitchell, MD**, **Joseph A. Vita, MD**, **Douglas B. Sawyer, MD**, and **Ramachandran S. Vasan, MD**

Framingham Heart Study, Framingham, MA (WL, JZ, EJB, MGL, RSV); Department of Cardiology, Children's Hospital Boston, Department of Pediatrics, Harvard Medical School, Boston, MA (JZ); Cardiovascular Division, Vanderbilt University, Nashville, TN (HMS, DBS), Cardiovascular Engineering Inc, Norwood, MA (GFM); Departments of Biostatistics (VX, LMS, QY) and Epidemiology (EJB), Boston University School of Public Health; Division of Graduate Medical Sciences (RS); Department of Mathematics (MGL); Department of Neurology (MHC), School of Medicine (EJB, JAV, RSV); Section of Preventive Medicine and Epidemiology (RSV), Boston University, Boston, MA

Abstract

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 Framingham Third Generation cohort participants (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 lower in women as compared to men. Ang-2 was positively related to age, smoking, systolic blood pressure (BP), hypertension treatment and diabetes ($p<0.05$ for all) but was inversely associated with total cholesterol and diastolic BP ($p<0.0001$ for both). Soluble Tie-2 was positively associated with body mass index, diabetes and triglycerides, but 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 BP 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 (LOD score 8.31).

Conclusion—Circulating levels of Ang-2 and sTie-2 are heritable traits that are associated with cardiovascular (CVD) risk factors including the metabolic syndrome. These observations are consistent with the notion that angiogenesis and vascular remodeling are in part determined by genetic influences, and associated with metabolic risk factors.

Address for correspondence: Ramachandran S. Vasan, MD, Framingham Heart Study, 73 Mount Wayte Ave, Framingham, MA 01702-5803, Tel. 508-935-3450, Fax: 508-626-1262, vasan@bu.edu.
*denotes equal contribution

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Keywords

Angiopoietins; vascular remodeling; heritability; metabolic syndrome; CVD risk factors

Introduction

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 is, therefore, critical for preventing CVD. The endothelium plays a central role in vascular remodeling, in part via endothelium-derived signaling proteins and receptors that mediate intercellular communication.1›2 Angiopoietins are an important group of endothelial growth factors that modulate angiogenesis,3 as well as vascular remodeling.4 While the various angiopoietins bind to the same endothelial tyrosine kinase receptor, Tie-2 (TEK), 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 signalling.7 Preformed Ang-2 in the Wiebel 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 soluble form of the Tie-2 receptor (sTie-2) has been described. 8 *SolubleTie*-2 downregulates endothelial Ang/Tie-2 signalling by binding to circulating angiopoietins and reducing the pool of free angiopoietins. Thus, a complex interplay of Ang-1, Ang-2, Tie-2, sTie-2, VEGF, and other pro- or anti-angiogenic factors determines progression of angiogenesis and vascular remodeling.

In smaller clinical studies, altered circulating concentrations of Ang-2 and/or Tie-2 were observed in patients with peripheral artery disease,[9] acute coronary syndrome,[10] and heart failure11 and reported to predict incident myocardial infarction.12 Furthermore, level of these biomarkers were associated with measures of subclinical disease13 and target organ damage in hypertension.[14] While these preliminary data suggest an important role for angiopoietins along the CVD continuum, the clinical and genetic correlates of Ang-2 and the soluble form of its receptor Tie-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 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 4,095 participants with at least one parent in the Offspring cohort were included. The data for the present analyses were obtained at the first examination cycle.[15] Participants were comprehensively evaluated, including anthropometric measurements, biochemical assessment for traditional CVD risk factors, and a medical history and physical examination by a Heart Study physician. The present 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), 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 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 inter-assay coefficients of variation were 5.7% for Ang-2 and 3.2% for sTie-2. Vascular endothelial growth factor A (VEGF), its soluble receptor sFlt-1, and hepatocyte growth factor (HGF) were assayed as previously reported.[16]

Electrocardiography

Presence of electrocardiographically determined left ventricular hypertrophy (LVH) was determined based on a standard 12-lead ECG when at least one of the following criteria were fulfilled:[17] Precordial RV5 or 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; 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 ($p \leq 0.1$ 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, estimated glomerular filtration rate [eGFR]). Then, we used generalized estimating equations (GEE; using 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 mmHg or antihypertensive medication); obesity (BMI≥30 kg/m^2); abdominal obesity (waist circumference ≥102 cm in men or ≥88 cm in women); low HDL cholesterol (<40 mg/dL in men or <50 mg/dL in women); high triglycerides (≥150 mg/dL or lipid-lowering medication) and the metabolic syndrome (defined as the presence of least 3 of the following features: increased waist circumference [see above]; elevated BP [≥130/85 mmHg, or anti-hypertensive treatment]; hyperglycaemia [fasting glucose ≥100 mg/dL or treatment for elevated glucose]; hypertriglyceridaemia [≥150 mg/dL or lipid-lowering treatment]; low HDL cholesterol [as defined above]).[18]

We evaluated the pair-wise correlations of Ang-2, and sTie-2 with other vascular growth factors measured in this cohort, i.e., VEGF, sFlt-1 and HGF[16] using Pearson's 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 were additionally 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 heritabilities for log-biomarker levels using two models; model 1 adjusted for age and sex, while model 2 additionally adjusted for all significant clinical correlates of the respective biomarker that were identified in the previous analyses detailed above.

Data from 3768 (10 participants of our sample lacked genotypic data) individuals belonging 1796 core families (online table 1) which constitute 805 pedigrees were used for a multipoint linkage analysis for Ang-2 and sTie-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 additionally 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.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

The clinical and biochemical characteristics of our sample are provided in Table 1. Our sample was comprised of young–to-middle-aged and included slightly more women than men. Information about the number of siblings per family is provided in online Table 1. Circulating concentrations of Ang-2 and sTie-2 were not correlated (r=0.004; p=0.3 in age- and sex-adjusted models). Pair-wise age- and sex-adjusted correlations between Ang-2, sTie-2, VEGF, sFlt-1, and HGF are provided in online Table 2. 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 Angiopoietin-2 and soluble Tie-2

Ang-2 levels were higher in women as compared to 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 inter-individual variation in circulating Ang-2 concentrations. We observed a positive association with hypertension when we replaced systolic and diastolic BP with the binary variable (β: 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 two measures with pulse pressure and observed a positive correlation between Ang-2 and pulse pressure (β: 0.028; 95% CI, 0.008 to 0.037 per 1 SD-increment; p=0.002). Replacing all significant continuous traits that are part of the definition of the metabolic syndrome, Ang-2 was also positively associated with the metabolic syndrome as a binary trait (β: 0.048; 95% CI, 0.015 to 0.082; p=0.0047). Ang-2 levels were also related to ECG-LVH (prevalence 8.1% in our sample; OR: 1.02; 95% CI, 1.01 to 1.04; p=0.011 per one-unit increment in log-Ang-2 levels (a 2.72-fold [e^1]-fold increment in Ang-2 in natural units).

Circulating sTie-2 concentrations were higher in men than 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 (β:0.046; 95% CI, 0.025 to 0.068; p<0.0001; indicating about 5% [$e^{0.046}$] higher Tie-2 levels in obese as compared to non-obese participants), waist circumference (β: 0.016; 95% CI, 0.005 to 0.026; p=0.004), abdominal obesity (β: 0.029; 95% CI, 0.01 to 0.049; p= 0.003) and the metabolic syndrome (β: 0.047; 95% CI, 0.025 to 0.068; p<0.0001). Soluble Tie-2 concentrations were not significantly associated with electrocardiographically determined LVH (OR: 1.00 95% CI, .097 to 1.03; p=0.95).

Genetic correlates of Angiopoietin-2 and soluble Tie-2

Heritability estimates were moderate for Ang-2 (27%) and substantial for sTie-2 (56%) in age- and sex-adjusted and in multivariable-adjusted models (Table 3). Multipoint LOD scores from the genome-wide linkage analysis for Ang-2 and sTie-2 levels are displayed in online Figure 1 and 2. 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 of 3). For sTie-2, the linkage peak (LOD score 8.31) was identified at 60 cM on chromosome 9 with two genotyped markers (GATA5E06 at 58.26 cM and GATA71E08 at 58.27 cM) nearby. The wide 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 almost 4000 individuals.

Principal findings

First, a sexual dimorphism for both biomarker concentrations was observed, with Ang-2 levels being significantly higher and sTie-2 concentrations significantly lower in women as compared to men. Relations to age were also opposite in directionality for the two markers, with Ang-2 increasing with age, but sTie-2 declining with age. Second, both Ang-2 and sTie-2 were positively related to diabetes and to the presence of the metabolic syndrome. However, the two biomarkers were associated with different components of the 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. Third, 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).

In the context of the literature

Sexual dimorphism in circulating Angiopoieting-2 and Tie-2 concentrations— Gender differences in vascular function have been well described[19] 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.[16,20] 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 e.g. of Ang-1,21 and VEGF22. Recent evidence suggest that also sTie-2 and Ang-2 levels were modulated by estrogen.[23] In agreement with our observation of higher Ang-2 levels in women (as compared to men), estrogen induces Ang-2 expression in several tissues.[23] 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 Angiopoietin-2

Circulating Ang-2 concentrations displayed 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 two previous clinical studies.[24,

[25] Increased levels of Ang-2 have been implicated in the development of diabetic microvascular complications including retinopathy.[26] Ang-2 and VEGF play an important role in the initiation of neovascularization[6] 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.[7] 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,[14] correlated with target organ damage[14] and predicted the incidence of myocardial infarction.[12] 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 [2,7] Several potential explanations can be invoked for the positive relations of Ang-2 to 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.27 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 anti-inflammatory and antiatherogenic properties,[28,29] and that COMP (cartilage oligomeric matrix protein)-Ang-1, a derivative of Ang-1, prevents the development of hypertension and associated target organ damage in pre-hypertensive spontaneously hypertensive rats.[30] Therefore, reduced Ang-1/Tie-2 activity could be causally related to the development of elevated BP. In this context, Ang-2 has also been reported to be an autocrine regulator of endothelial cell responses to inflammatory stimuli, with a permissive role for the effects of proinflammatory cytokines.31 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 to non smokers), consistent with heightened stress-induced senescence of endothelial cells in smokers.[32]

Our finding of an association between Ang-2 and ECG-determined LVH are consistent with angiogenesis being necessary for cardiac hypertrophy but are also in good agreement with recent basic science data suggesting anti-hypertrophic effect of Ang-1 on cardiac myocytes through binding to integrin receptors.[33] As detailed above, 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

Soluble Tie-2 concentrations displayed highly significant associations with the metabolic syndrome and its components, including diabetes, 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 study34 and agree well with the positive association of other endothelial growth factors, including HGF, with obesity and the metabolic syndrome.16 Given the strong interdependence of the adipose tissue and angiogenesis, this positive association between sTie-2, which is the key endothelial receptor for all angiopoietins,[2] and the metabolic syndrome and obesity (including abdominal obesity) is not surprising. The adipose tissue is well vascularized,[35] and the growth of adipose tissue requires the presence of angiogenic factors and their receptor. It has indeed been shown in mouse models of obesity that Ang-2 expression was increased in subcutaneous adipose

tissue36 and that inhibitors of angiogenesis effectively reduced weight and adipose tissue mass. 37 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 producing a broad array of hormones, including promoters and inhibitors of angiogenesis.35 It is not clear what the presence of *soluble* Tie-2 indicates in this context. While 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 if the positive association of sTie-2 with BMI is due to increased elaboration of the growth factor by the expanded fat compartment, or if it is in response to the greater need for angiogenesis as the compartment expands.Tie-2 is expressed in glomerular capillary endothelial cells.[38] 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.[39] Alternatively, lower eGFR may be associated with greater Tie-2 expression.[40] The association with alcohol intake is consistent with experimental observations that ethanol increases angiogenesis via a Tie-2-dependent pathway.[41]

Genetic correlates of Angiopoietin-2 and sTie-2

We observed that 56% of the variation in circulating sTie-2 concentrations was due to genetic factors. Ang-2 levels had a moderate heritability of 27%. This is the first study 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 factors16 and support the notion that circulating levels of angiogenic peptides are in part genetically determined. This concept is further supported by experimental studies reporting that expression of Ang-1, sTie-2 and other angiogenic biomarkers in response to chronic hypoxia differed between mouse strains of varied genetic background.[42] 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, that is expressed in macrophages and has been suggested to play a role in the development of atherosclerotic lesions.[43] Furthermore, the *NPR2* 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 protein family, which has recently been implicated in cardiac hypertrophy.[44]

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. 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 that were not in strong linkage disequilibrium with one of the 687 genetic markers we used. The cross-sectional design precludes any causal interferences. Our sample is young-to-middle-aged, and 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 these observations are clinically significant.

Conclusion

Circulating concentrations of soluble Tie-2 and Ang-2 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics of our study sample.

	Women (n=2018)	Men (n=1760)
Age, years	40±9	40±9
Systolic BP, mm Hg	113±14	121±12
Diastolic BP, mm Hg	73±9	78±9
Hypertension, %	12	20
Poorly controlled Hypertension, %	2.0	2.8
Well controlled Hypertension, %	5.3	6.2
BMI, kg/m^2	25.9±6.0	27.9±4.7
Waist circumference, cm	86.9±15.3	95.5±12.5
Obesity, %	20	25
Abdominal obesity, %	40	34
Current smoker, %	14	16
Alcohol consumption, ounces per week	3 (0,8)	8 (2,19)
eGFR, ml/min/1.73m^2	99±32	100±17
Glucose, mg/dL	92±18	98±16
Diabetes, %	2.3	3.0
Insulin-dependent Diabetes (% of participants with diabetes)	23	10
UACR, mg/g	4.7 (3.1,8.9)	3.2 (2.2,5.8)
Total cholesterol, mg/dL	185±34	193±37
LDL cholesterol, mg/dL	105±30	120±32
HDL cholesterol, mg/dL	61±16	47±12
Triglycerides, mg/dL	80 (59,114)	107 (72,161)
Metabolic Syndrome, %	15	28
Dyslipidemia, %	29	40
Family history of CVD, %	33	33
Angiopoietin-2, ng/mL	2.03 (1.51,2.72)	1.71 (1.34,2.23))
Log-Angiopoietin-2, ng/mL	0.71 (0.41,1.00)	0.54 (0.29,0.80)
Soluble Tie-2, ng/mL	14.35 (12.02,17.42)	15.02 (12.71,18.29)
Log-sTie-2, ng/mL	2.71 (2.54,2.91)	2.66 (2.49,2.86)

BP, blood pressure; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate, BMI, body mass index; eGFR, estimates glomerular filtration rate; UACR, urinary albumin/creatinine ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; . Data are mean±standard deviation for continuous traits (except for triglycerides, Angiopoietin-2, Tie-2 and alcohol consumption where median (quartile1, quartile3) are provided) and percentages for categorical data.

Definitions: poorly controlled hypertension: participants on antihypertensive medication, but with systolic BP ≥140 or diastolic BP ≥ 90 mmHg; well controlled hypertension: participants on antihypertensive medication and with a systolic BP <140 and a diastolic BP <90 mmHg; obesity: body mass index ≥ 30 kg/m^2); abdominal obesity: waist circumference ≥ 89 cm in women and ≥ 102 cm in men; dyslipidemia: total cholesterol > 200 mg/dL; smoking: smoking at least on cigarette per day in the year preceding the examination.

Table 2

Clinical correlates of Angiopoietin-2 and soluble Tie-2 as determined by linear regression models.

	Increase/decrease in log-Biomarker level			Percent increase/decrease in biomarker in natural units			
	95% confidence interval			95% confidence interval			
Angiopoietin-2	**Estimate**	**Lower Limit**	**Upper Limit**	**Estimate**	**Lower Limit**	**Upper Limit**	**p-value**
Age, years	0.019	0.003	0.035	1.92%	0.30%	3.56%	0.02
Men	−0.149	−0.178	−0.12	−13.84%	−16.31%	−11.31%	<.0001
Smoking	0.159	0.119	0.198	17.23%	12.64%	21.90%	<.0001
Total cholesterol, mg/dL	−0.037	−0.051	−0.023	−3.63%	−4.97%	−2.27%	<.0001
Antihypertensive Medication	0.091	0.04	0.142	9.53%	4.08%	15.26%	0.0004
Diastolic blood pressure, mm Hg	−0.045	−0.064	−0.025	−4.40%	−6.20%	−2.47%	<.0001
Systolic blood pressure, mm Hg	0.031	0.011	0.051	3.15%	1.11%	5.23%	0.003
Diabetes	0.097	0.001	0.192	10.19%	0.10%	21.17%	0.048
Soluble Tie-2							
Age, years	−0.045	−0.056	−0.034	−4.40%	−5.45%	−3.34%	<.0001
Men	0.043	0.024	0.062	4.39%	2.43%	6.40%	<.0001
Body mass index, kg/m^2	0.016	0.006	0.027	1.61%	0.60%	2.74%	0.002
Diabetes	0.102	0.04	0.164	10.74%	4.08%	17.82%	0.001
eGFR, ml/min/1.73m^2	−0.017	−0.027	−0.007	−1.69%	−2.66%	−0.70%	0.0005
Alcohol consumption	−0.011	−0.023	−0.0004	−1.09%	−2.27%	−0.04%	0.04
Triglycerides, mg/dL	0.012	0.003	0.021	1.21%	0.30%	2.12%	0.01

The first three columns describe the estimates (and 95% CI) representing the increase in log-biomarker levels per 1-SD increment in the predictor variable for continuous traits. For binary traits, estimates indicate increase in log-biomarker levels for presence vs. absence of the trait. For example, men have 14% lower ($e^{-0.149}$ =0.86) Ang-2 levels as compared to women, adjusting for all other covariates in the model. In the next three columns, the % change in biomarker levels in natural units per 1-SD increment in the predictor variable (presence vs. absence of binary traits) is shown. The multivariable model explained 6.8% and 3.8% of the inter-individual variation in circulating Ang-2 and sTie-2 concentrations, respectively.

* Alcohol consumption in ounces per week.

Table 3

Heritability estimates for Angiopoietin-2 and soluble Tie-2

Biomarker	Model	Heritability	95% CI	p-value
Angiopoietin-2	Age- and sex-adjusted	0.28	(0.21,0.35)	3.9×10^{-17}
	multivariable-adjusted	0.27	(0.19,0.34)	1.7×10^{-16}
Soluble Tie-2	Age- and sex-adjusted	0.55	(0.47,0.62)	1.2×10^{-57}
	multivariable-adjusted	0.56	(0.48,0.63)	5.2×10^{-59}

Multivariable-adjusted models adjusted for those covariates that were significantly related to Angiopoietin-2 and Tie-2, respectively in Table 2.