

## Recent Advances in the Development of Cardiovascular Biomarkers

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Cardiovascular diseases (CVD) are initiated by endothelial dysfunction and resultant expression of adhesion molecules for inflammatory cells.<sup>1-3</sup> Inflammatory cells secrete cytokines/chemokines and growth factors and promote CVD.<sup>4</sup> Additionally, vascular cells themselves produce and secrete several factors, some of which can be useful for the early diagnosis and evaluation of disease severity of CVD.<sup>5-7</sup> Among vascular cells, abundant vascular smooth muscle cells (VSMCs) secrete a variety of humoral factors that affect vascular functions in an autocrine/paracrine manner.<sup>8,9</sup> Among these factors, we reported that CyPA (cyclophilin A) is secreted mainly from VSMCs in response to Rho-kinase activation and excessive reactive oxygen species (ROS).<sup>10-12</sup> Additionally, extracellular CyPA augments ROS production, damages vascular functions,<sup>13</sup> and promotes CVD.<sup>8,14-24</sup> Importantly, a recent study in *ATVB* demonstrated that ambient air pollution increases serum levels of inflammatory cytokines.<sup>25</sup> Moreover, Bell et al<sup>26</sup> reported an association of air pollution exposure with high-density lipoprotein (HDL) cholesterol and particle number. In a large, multiethnic cohort study of men and women free of prevalent clinical CVD, they found that higher concentrations of PM<sub>2.5</sub> over a 3-month time period was associated with lower HDL particle number, and higher annual concentrations of black carbon were associated with lower HDL cholesterol. Together with the authors' previous work on biomarkers of oxidative stress, they provided evidence for potential pathways that may explain the link between air pollution exposure and acute cardiovascular events.<sup>25,26</sup> The objective of this review is to highlight the novel research in the field of biomarkers for CVD.

### Biomarkers for Atherosclerosis (Coronary Artery Disease)

Endothelial dysfunction induces the development of atherosclerotic disease.<sup>3,27,28</sup> In the maintenance of endothelial function and vascular homeostasis, AMPK (AMP-activated protein kinase) and its downstream signaling of endothelial NO synthase play crucial roles in regulating the appropriate levels of blood pressure.<sup>29</sup> In contrast, risk factors for

atherosclerosis, such as hypertension, dyslipidemia, smoking, and diabetes mellitus, deteriorate endothelial function, upregulate adhesion molecules, induce inflammatory cell migration, and activate VSMCs, promoting the development of atherosclerosis.<sup>11,12</sup> One common mechanism for the deterioration of vascular function is augmented ROS production in vascular tissues.<sup>10,12,30</sup> As mentioned above, we previously demonstrated that CyPA can be secreted by oxidative stress, and extracellular CyPA stimulates endothelial cells to induce adhesion molecules,<sup>17</sup> apoptosis,<sup>21</sup> and inflammatory cell migration.<sup>9</sup> Moreover, *in vivo* studies demonstrated that CyPA promotes the development of intimal thickening,<sup>17</sup> abdominal aortic aneurysms,<sup>19</sup> atherosclerosis,<sup>21</sup> cardiac hypertrophy,<sup>22</sup> and pulmonary hypertension (PH).<sup>23</sup> Further translational research showed that plasma levels of CyPA were significantly increased in patients with coronary artery disease (CAD).<sup>8</sup> Thus, circulating CyPA can be useful as a novel biomarker for patients with CAD.<sup>12</sup> Mechanistically, CyPA participates in lipid uptake by affecting scavenger receptors during fatty streak formation.<sup>21</sup> Additionally, the incidence of all-cause death, rehospitalization, and revascularization was higher in patients with higher plasma CyPA levels than in those with lower plasma levels.<sup>24</sup> In this study, combining other biomarkers, such as hsCRP (high-sensitivity C-reactive protein) and BNP (brain natriuretic peptide), further enhanced the prognostic impacts of CyPA in patients with CAD. Conversely, several biomarkers have been reported in recent publications in *ATVB* (Table 1). Chen et al<sup>31</sup> reported that lower plasma levels of fetuin-A ( $\alpha$ -2-AHSG [ $\alpha$ -2-heremans-schmid glycoprotein]) are associated with a higher mortality risk in patients with CAD. Fetuin-A is the most important inhibitor of calcification and a factor linked with cardiovascular mortality.<sup>31</sup> Here, they revealed that patients with higher plasma fetuin-A levels had lower risks of all-cause and CVD mortality.<sup>31</sup> In addition, Saita et al<sup>32</sup> reported that plasma soluble endoglin levels were inversely associated with the severity of CAD. Here, they reported that plasma soluble endoglin levels were low in patients with stable CAD, especially those with 3-vessel disease. Then, Caselli et al<sup>33</sup> evaluated the relative effects of both coronary atherosclerosis and myocardial ischemia on cardiac release of high-sensitivity cardiac troponin T and NT-proBNP (N-terminal pro-B-type natriuretic peptide) in patients with stable CAD. In this study, they showed that circulating levels of high-sensitivity cardiac troponin T were related to the presence and extent of coronary lesions and coronary plaque composition. Moreover, circulating levels of NT-proBNP were mainly related with the presence of functionally relevant coronary disease that causes myocardial ischemia.<sup>33</sup> Conversely, Gill et al<sup>34</sup> reported the effect of iron status on risk of CAD. In this study, the authors showed a protective effect of higher iron levels on the risk of CAD. Finally, Doi et al<sup>35</sup> reported

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(*Arterioscler Thromb Vasc Biol*. 2018;38:e61-e70.  
DOI: 10.1161/ATVBAHA.118.310226.)

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*Arterioscler Thromb Vasc Biol* is available at <http://atvb.ahajournals.org>  
DOI: 10.1161/ATVBAHA.118.310226

**Table 1. Summary of Biomarkers in Vascular Inflammation**

Types of Vascular Diseases	Biomarker	Reference
Atherosclerosis (CAD)	Cyclophilin A	8,24
	Fetuin-A	31
	Soluble endoglin	32
	Cardiac troponin T	33
	Iron status	34
	Coronary artery ectasia	35
	Rho-kinase	36–38
	Serotonin	39
Atherosclerosis (other than CAD)	Endogenous cholesterol excretion	42
	mtDNA damage and dysfunction	43
	Sortilin	44
	High-mobility group box 2	46
	Ceramides	48
	Total bilirubin	49
	Ferritin	50
Pulmonary arterial hypertension	Cyclophilin A	23
	Acetylated cyclophilin A	64
	Basigin	23
Other vascular inflammation	vWF and ADAMTS13	71
	Fibroblast growth factor 12	72
	ANGPTL2	73
	IL-1RA	75
	Uric acid and acute-phase reactants	76
	Fatty acid-binding protein 4	77
	Retinol-binding protein 4	77
	High-molecular-weight adiponectin	77
	FADD	78
	Caspase-3	78
	Caspase-8	78

ADAMTS13 indicates a disintegrin and metalloproteinase with thrombospondin motifs 13; ANGPTL2, angiotensin-converting enzyme 2; CAD, coronary artery disease; FADD, Fas-associated death domain-containing protein; IL-1RA, interleukin-1 receptor antagonist; mtDNA, mitochondrial DNA; and vWF, von Willebrand factor.

that coronary artery ectasia predicts future cardiac events in patients with acute myocardial infarction. They demonstrated that 3% of patients with acute myocardial infarction harbored coronary artery ectasia. Moreover, patients with coronary artery ectasia more frequently developed nonfatal myocardial infarction and cardiac death.<sup>35</sup> Thus, they concluded that coronary artery ectasia is an important morphological characteristic of CAD that causes cardiac events. In addition to these biomarkers in CAD, we have demonstrated the importance of several biomarkers, including Rho-kinase for vasospastic angina<sup>36–38</sup> and serotonin for microvascular angina.<sup>39</sup>

## Biomarkers for Atherosclerosis (Other Than CAD)

Key mechanisms in abdominal aortic aneurysm formation include VSMC senescence, oxidative stress, local proinflammatory cytokine production, and increased matrix metalloproteinase activity, all of which degrade the extracellular matrix.<sup>19</sup> Here, CyPA also plays a crucial role in the development of aortic aneurysms and can be a novel therapeutic target.<sup>12,40</sup> Besides CyPA, several factors have been proposed as possible biomarkers for types of atherosclerosis other than CAD. Leung et al<sup>41</sup> reported that blood pressure and heart rate measures were associated with increased risk of silent cerebral infarction and worsening leukoaraiosis in elderly adults. This study provided evidence that systolic hypertension is associated with increased risk for silent cerebral infarction and diastolic hypertension is associated with increased risk for leukoaraiosis. Thus, these findings suggest a need to reevaluate the balance of benefits and risks of controlling systolic and diastolic hypertension in elderly adults. Then, Lin et al<sup>42</sup> reported that endogenous cholesterol excretion is negatively associated with carotid intima-media thickness in humans. The authors showed that fecal excretion of endogenous cholesterol (the principal pathway of cholesterol elimination in humans) is negatively correlated with carotid intima-media thickness. Additionally, Yu et al reported that mitochondrial respiration is reduced in atherosclerosis, promoting necrotic core formation and reducing relative fibrous cap thickness. This study suggested that preventing the mitochondrial DNA damage and dysfunction seen in atherosclerosis is important in modulating plaque composition. Conversely, Nguyen et al<sup>43</sup> reported that extracellular vesicles secreted by atherogenic macrophages transfer microRNA to inhibit cell migration. Moreover, Heffron et al<sup>44</sup> showed that greater frequency of fruit and vegetable consumption is associated with lower prevalence of peripheral artery disease. Their adjusted analyses demonstrated a stepwise reduction in odds for peripheral artery disease with increasing consumption of fruit and vegetable. Notably, this association was restricted to people who were current or former cigarette smokers.<sup>44</sup> Goettsch et al<sup>45</sup> reported that serum levels of sortilin are associated with aortic calcification and cardiovascular risk in men. Their study demonstrated that high serum sortilin levels associate with increased risk for major adverse cardiac event and stroke after adjustment for multiple relevant confounders, including CRP, total cholesterol, and low-density lipoprotein (LDL) cholesterol in older men, suggesting a cholesterol-independent role of sortilin in CVD.<sup>45</sup> Conversely, He et al<sup>46</sup> reported that serum levels of HMGB2 (high-mobility group box 2) are associated with in-stent restenosis. They demonstrated an association between HMGB2 and in-stent restenosis in patients. Furthermore, Small et al<sup>47</sup> recently reported on the biomarkers of calcific aortic valve disease. Potential use of such biomarkers in calcific aortic valve disease includes screening for early aortic valvular disease and identifying patients who are most likely to progress to severe disease. Havulinna et al<sup>48</sup> reported that circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. The ceramide analysis of the FINRISK cohort showed that distinct ceramide

species are associated with major cardiovascular events at the population level. The relatively strong univariate associations of ceramides with fatal events suggested that ceramides may play a role in the rupture of atherosclerotic plaques.<sup>48</sup> Amor et al<sup>49</sup> reported a relationship between total serum bilirubin levels and carotid and femoral atherosclerosis in familial dyslipidemia. This study showed that, in participants with familial dyslipidemia, total bilirubin levels were inversely associated with carotid and femoral atherosclerosis, independent of other classical and nonclassical cardiovascular risk factors or hypolipidemic drug treatment. Finally, Prats-Puig et al<sup>50</sup> reported that serum levels of ferritin are related with carotid intima-media thickness in the offspring of fathers with higher serum ferritin levels. The findings of this study indicate that circulating ferritin levels are associated with carotid intima-media thickness in healthy children. Furthermore, the association between the level of ferritin in children and the change in carotid intima-media thickness was highly significant in children whose fathers had higher ferritin levels.<sup>50</sup>

### Biomarkers for Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is categorized as group I of PH World Health Organization clinical classification system. In addition to genetic backgrounds, environmental factors are substantially involved in the development of PAH.<sup>51</sup> Pulmonary artery smooth muscle cells (PASMCs) of patients with PAH have the unique characteristic of excessive proliferation, which is markedly different from those of healthy controls. Pulmonary artery endothelial cells contribute to pulmonary vascular homeostasis, and deterioration initiates the development of PAH.<sup>7,51,52</sup> Indeed, we have revealed a protective role of the endogenous Epo (erythropoietin)/EpoR (Epo receptor) system against the development of PH.<sup>14</sup> This system also plays a crucial role in the functional recovery of ischemic heart<sup>15</sup> and ischemic lower limb,<sup>16</sup> demonstrating the importance of endothelial function and homeostasis.<sup>53,54</sup> However, excessive Epo (exogenous Epo administration) did not show beneficial effects, with conflicting results among several CVD studies.<sup>55–57</sup> These conflicting results may suggest the opposing mechanistic roles of Epo in the cardiovascular system.<sup>58</sup> Here, Epo production is regulated by hypoxia-inducible factor-1- $\alpha$ , which also regulates the transcription of many other proteins.<sup>30</sup> Moreover, the Epo-mediated increase in circulating erythrocytes enhances oxygen supply but results in higher oxidative stress.<sup>30</sup> Oxidative stress induces endothelial dysfunction, PASMC proliferation, and vascular inflammation, all of which promotes the development of PAH.<sup>30,59–61</sup> As mentioned before, extracellular CyPA augments oxidative stress in cardiovascular tissue.<sup>10</sup> Rho-kinase has a crucial role in the secretion of CyPA from VSMCs.<sup>4,7</sup> Moreover, Rho-kinase was substantially involved in the development of PH in mice and humans.<sup>51,60–62</sup> Indeed, Karoor et al<sup>63</sup> reported that sustained activation of Rho GTPases promotes a synthetic PASMC phenotype in neprilysin knockout mice. Moreover, extracellular CyPA and its receptor, Bsg (basigin), played a crucial role in hypoxia-induced PH by inducing growth factor secretion, inflammation, and VSMC proliferation.<sup>23</sup>

Importantly, a recent study in *ATVB* by Xue et al<sup>64</sup> further demonstrated that extracellular CyPA, especially acetylated CyPA, causes PH by stimulating endothelial apoptosis, redox stress, and inflammation. Clinically, plasma CyPA levels were significantly elevated and predicted poor outcomes in patients with PAH.<sup>23</sup> Besides CyPA, recent studies have revealed novel pathogenic proteins that can be useful biomarkers for patients with PAH.<sup>65–67</sup> Additionally, the development of imaging techniques enabled a more precise evaluation of pulmonary arterial remodeling in patients with PAH.<sup>68,69</sup> Moreover, drug repositioning is the process of discovering, validating, and marketing previously approved drugs for new indications. Here, we found that pravastatin and metformin ameliorate hypoxia-induced PH in animals.<sup>18,52</sup> Conversely, Meloche et al<sup>70</sup> showed inflammation and epigenetic readers in coronary artery remodeling in patients with PAH. Importantly, they explored coronary abnormalities at the cellular and molecular level in patients with PAH. Interestingly, they observed that BRD4 (bromodomain-containing protein 4), which is increased in the lungs of patients with PAH, is strongly expressed in their coronary arteries.<sup>70</sup> Moreover, they showed that the coronary arteries of patients with PAH had greater wall thickness, exhibited increased inflammation, sustained DNA damage, and had significantly more BRD4-expressing cells.

### Biomarkers for Vascular Inflammation and Growth Factors

There are several important publications on novel biomarkers in the recent issues of *ATVB*. First, Sonneveld et al<sup>71</sup> reported the effect of vWF (von Willebrand factor) and a disintegrin and metalloproteinase with a thrombospondin motifs 13 (ADAMTS13) on the risk of mortality. In this large prospective cohort study, ADAMTS13 activity and vWF:Ag levels were associated with an increased risk of mortality, especially cardiovascular mortality. These risks were independent of prevalent CVD and established cardiovascular risk factors. Additionally, Song et al<sup>72</sup> reported that FGF12 (fibroblast growth factor 12) is a novel regulator of VSMC plasticity and fate. In this study, they identified FGF12 as a novel master regulator of VSMC plasticity and fate. By performing both in vitro and in vivo experiments, they observed that FGF12 was necessary and sufficient for inducing phenotype switching of VSMCs into the quiescent and contractile states.<sup>72</sup> Additionally, Hata et al<sup>73</sup> reported that serum ANGPTL2 (angiopoietin-like protein 2) is a novel risk factor for CVD in the community (the Hisayama study). They demonstrated that the risk for the development of CVD increased significantly with elevating serum ANGPTL2 levels. Januzzi et al<sup>74</sup> reported the association between circulating proneurotensin concentrations and CVD events in the community (the Framingham Heart Study). The principal findings of this analysis were that concentrations of proneurotensin were associated cross-sectionally with a more deleterious cardiometabolic phenotype, and they were prospectively associated with incident cardiovascular events in the population, independently of LDL concentrations.

Then, Herder et al<sup>75</sup> reported on circulating levels of IL-1RA (interleukin-1 receptor antagonist) and risk of CVD.

Their study showed that CVD risk increased by 11% per 1-SD increase in serum IL-1RA based on data from >20 000 study participants from 6 population-based cohort studies. Additionally, the association between IL-1RA and CVD risk was partially explained by biomarkers of subclinical inflammation, oxidative stress, and cell adhesion.<sup>75</sup> Spiga et al<sup>76</sup> also reported that uric acid is associated with inflammatory biomarkers and induces inflammation via activation of the NF- $\kappa$ B (nuclear factor- $\kappa$ B) signaling pathway in HepG2 cells. In this study, they reported a positive relationship between serum uric acid and acute-phase reactants, such as hsCRP, fibrinogen, ferritin, complement C3, and erythrocyte sedimentation rate. Importantly, the relationship remained significant after adjustment for several confounders potentially affecting either serum uric acid or inflammatory biomarkers levels, including age, sex, adiposity, antihypertensive treatments, and diuretic use. Importantly, they provided evidence that uric acid induces a proinflammatory effect through the NF- $\kappa$ B signaling pathway.<sup>76</sup> Moreover, Liu et al<sup>77</sup> reported on the role of plasma levels of FABP4 (fatty acid-binding protein 4), RBP4 (retinol-binding protein 4), and high-molecular-weight adiponectin on cardiovascular mortality in men with type 2 diabetes mellitus. In their prospective study, they found a significant association between elevated levels of FABP4 and increased total and CVD mortality. Finally, Xue et al<sup>78</sup> reported the role of FADD (fas-associated death domain-containing protein), caspase-3, and caspase-8 for the incidence of coronary events. They demonstrated that activation of apoptosis signaling through the Fas receptor is associated with a release of the intracellular apoptosis signaling components FADD, caspase-8, and caspase-3 into the extracellular compartment, suggesting that they represent possible biomarkers of apoptotic activity. In a population-based cohort, they demonstrated an association between the incidence of coronary events and high levels of FADD and caspase-8, but not caspase-3, at baseline levels. They concluded that an increased rate of apoptosis, as represented by a high expression of FADD and caspase-8 in the blood, was associated with an increased incidence of coronary events.<sup>78</sup>

### Biomarkers for Lipid Metabolism

In the field of lipid metabolism, there are several important recent publications in the *ATVB* journal (Table 2). First, Moriarty et al<sup>79</sup> reported that lipoprotein(a) mass levels increase significantly according to *APOE* genotype. They documented that *APOE* isoforms strongly influence lipoprotein(a) mass levels, with a 65% increase in  $\epsilon 4/\epsilon 4$  compared with  $\epsilon 2/\epsilon 2$  genotypes. Consistent with this, lipoprotein(a) cholesterol levels were also increased according to *APOE* genotypes. Additionally, Bajaj et al<sup>80</sup> reported the associations between lipoprotein(a) and risk of myocardial infarction and death in patients with chronic kidney disease. Their study offered substantial evidence for the association between elevated lipoprotein(a) and adverse outcomes in a large and diverse cohort of patients with chronic kidney disease. Findings from the analyses also showed that, with increasing severity of renal disease at baseline, lipoprotein(a) levels were progressively higher.<sup>80</sup> Moreover, Kim et al<sup>81</sup> reported that variability in total cholesterol is associated

**Table 2. Summary of Biomarkers in Other Cardiovascular Systems**

Types of Cardiovascular Systems	Biomarker	Reference
Lipid metabolism	Lipoprotein(a)	79,80
	Variability in total cholesterol	81
	Subcutaneous adipocyte lipolysis	82
	MDA-LDL and apoB-immune complexes	83
	Apolipoprotein B-100 autoantibodies	84
	Anti-apoA-1 IgG	85
	IgM-p45MDA autoantibodies	84
	IgG-p210 native autoantibodies	84
	Apolipoprotein C-III	86
	ApoM/S1P complex	87
	Remnant cholesterol	88
	Non-HDL cholesterol/triglycerides	89
Cholesterol efflux capacity	91	
Coagulation and fibrinolysis system	TAFI	92,93
	Minor allele <i>CPB2</i>	92
	Factor IXa activity	102
	D-dimer	103
	STXBP5	104
Heart failure	BNP, NT-proBNP, ANP	105,106
	Rho-kinase	62,108
	Cyclophilin A	22
	Basigin	23,110
	Nonfasting triglycerides/LDL cholesterol	117

ANP indicates atrial natriuretic peptide; apoM, apolipoprotein M; BNP, brain natriuretic peptide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA-LDL, malondialdehyde-modified LDL; NT-proBNP, N-terminal pro-B-type natriuretic peptide; S1P, sphingosine-1-phosphate; STXBP5, syntaxin-binding protein 5; and TAFI, thrombin-activatable fibrinolysis inhibitor.

with the risk of end-stage renal disease. In this nationwide population-based cohort study, they demonstrated that total cholesterol variability was associated with a higher risk for end-stage renal disease development. The association persisted after multivariable adjustment for important potential confounders, including baseline estimated glomerular filtration rate, proteinuria, and underlying diseases. These results add to the evidence that total cholesterol variability is a risk factor for end-stage renal disease, as well as for myocardial infarction, stroke, and all-cause mortality.<sup>81</sup>

Rydén et al<sup>82</sup> reported that subcutaneous adipocyte lipolysis contributes to circulating lipid levels. This study sheds new light on the role of subcutaneous fat cell lipolysis in influencing circulating lipid levels. Furthermore, the influence of adipocyte lipolysis was only important for plasma HDL cholesterol and triglycerides. Importantly, the observed relationships were independent of classical risk factors influencing plasma lipids, such as sex, age, body mass index, body shape,

use of nicotine, treatment of hypertension, diabetes mellitus, or hyperlipidemia, and fat cell size.<sup>82</sup> Conversely, Prasad et al<sup>83</sup> reported an interesting relationship of autoantibodies to malondialdehyde-modified LDL and apoB-immune complexes to sex, ethnicity, subclinical atherosclerosis, and cardiovascular events. Additionally, Björkbacka et al<sup>84</sup> reported that low levels of apolipoprotein B-100 autoantibodies are associated with an increased risk of coronary events. Moreover, Antiochos et al<sup>85</sup> showed the impact of CD14 polymorphisms on antiapoprotein A-1 IgG-related CAD prediction in the general population. The main finding of their study is that anti-apoA-1 IgG is independently associated with nonfatal incident CAD in the general population. This Swedish prospective population-based study including 5393 white individuals currently represents the largest study investigating the role of autoantibodies recognizing apoB-100 peptides in CVD. Additionally, the IgM-p45 malondialdehyde-modified and the IgG-p210 native autoantibodies, respectively, demonstrated independent association with lower risk for future coronary events.<sup>84</sup> These recent studies determined that these autoantibody levels can predict risk of future cardiovascular events in individuals of the general community.

van Capelleveen et al<sup>86</sup> reported on apolipoprotein C-III levels and incident CAD risk. Their study demonstrated that elevated apolipoprotein C-III levels are associated with a significantly increased risk of CAD. Furthermore, the risk of elevated apolipoprotein C-III levels was strongly associated with several parameters of triglyceride-rich lipoprotein, small LDL particles, and inflammation. Frej et al<sup>87</sup> reported that a shift in apoM (apolipoprotein M)/sphingosine-1-phosphate between HDL particles in women with type 1 diabetes mellitus is associated with impaired anti-inflammatory effects of the apoM/sphingosine-1-phosphate complex. Here, they showed that light-density HDL particles are less anti-inflammatory than denser HDL particles in both patients with type 1 diabetes mellitus and healthy controls. In addition, they found that when the apoM/sphingosine-1-phosphate complex is located on light HDL particles, it loses its inhibitory effects on the expression of proinflammatory adhesion molecules on the endothelial surface. Finally, they concluded that patients with type 1 diabetes mellitus have increased amounts of lighter density HDL particles and hence have more ineffective HDL with respect to apoM/sphingosine-1-phosphate functionality.<sup>87</sup> Bernelot Moens et al<sup>88</sup> reported that remnant cholesterol elicits arterial wall inflammation and a multilevel cellular immune response in humans. Puri et al<sup>89</sup> reported the roles of non-HDL cholesterol and triglycerides in coronary atheroma progression and clinical events. Their study is the first to demonstrate that coronary disease progression is more tightly linked with changes in non-HDL cholesterol compared with those in LDL cholesterol and that on-treatment triglyceride levels are associated with coronary atheroma progression, especially when these levels exceed 200 mg/dL. Moreover, lowered non-HDL cholesterol and triglyceride levels significantly modulated plaque progression. They concluded that their findings provide mechanistic support for the possible roles of non-HDL cholesterol and triglycerides to more definitively emerge as future therapeutic targets, especially in statin-treated patients requiring secondary prevention.<sup>89</sup>

Kockx et al<sup>90</sup> reported on the LDLr (LDL receptor)-dependent and LDLr-independent mechanisms of cyclosporin A (CsA)-induced dyslipidemia. They demonstrated that CsA-induced dyslipidemia does not require inhibition of the LDLr or the presence of a high-fat Western diet. This study identified a complex interaction among CsA, lipoprotein clearance pathways, and the LDLr. Thus, LDLr deficiency seems to be permissive for CsA-induced hyperlipidemia. Treatment with CsA markedly increased plasma cholesterol and triglyceride levels in chow-fed *Ldlr*<sup>-/-</sup> mice, and this was associated with a marked increase in plasma very-low-density lipoprotein and intermediate-density lipoprotein/LDL levels, decreased lipolysis, hepatic lipoprotein clearance, and increased plasma PCSK9 (proprotein convertase subtilisin/kexin type 9) levels.<sup>90</sup> Finally, Ogura et al<sup>91</sup> reported an association between cholesterol efflux capacity and atherosclerotic CVD in patients with familial hypercholesterolemia. The main finding of their study was that decreased cholesterol efflux capacity was associated with increased risk of cardiovascular events, even in statin-treated patients with familial hypercholesterolemia. In addition, they found that patients with corneal arcus had lower cholesterol efflux capacity after adjustment for age and sex. Additionally, there was an inverse association of cholesterol efflux capacity with Achilles tendon thickness and carotid intima-media thickness after adjustment for age, sex, and traditional cardiovascular risk factors. However, these associations diminished after additional adjustment for HDL or ApoA-1.<sup>91</sup>

### Biomarkers for Coagulation and Fibrinolysis System

In addition to PAH, we have recently demonstrated that thrombin-activatable fibrinolysis inhibitor (TAFI) is a novel biomarker for patients with chronic thromboembolic PH (CTEPH).<sup>92,93</sup> CTEPH is categorized as group IV of PH by World Health Organization classification.<sup>94-97</sup> The emergence of balloon pulmonary angioplasty significantly improved the prognosis of patients with CTEPH, which is a serious disorder causing severe right ventricular failure and death.<sup>98-101</sup> The main feature of CTEPH is obstruction of pulmonary arteries by organized thrombi.<sup>94,98</sup> Because the pathogenesis of CTEPH has been unclear for a long time, we have attempted to find a key molecule to elucidate the pathogenesis of this disorder. Here, we found that plasma levels of TAFI were significantly elevated in CTEPH patients and were unaltered even after hemodynamic improvement.<sup>92,93</sup> Additionally, the levels of TAFI released from platelets were increased in CTEPH patients. Taken together, these results indicate that TAFI is substantially involved in the resistance to thrombus fibrinolysis in CTEPH patients. Additionally, we found the minor allele *CPB2* in CTEPH patients compared with general population.<sup>92</sup> Moreover, plasma levels of activated TAFI (aTAFI) were negatively correlated with clot lysis time in CTEPH patients.<sup>92</sup> Thus, to evaluate the effects of aTAFI inhibition, we performed *in silico* screening using the Life Science Knowledge Bank database and found several aTAFI inhibitors, and one of them ameliorated the development of PH in mice.<sup>93</sup> Additionally, we found that PPAR- $\alpha$  (peroxisome proliferator-activated receptor- $\alpha$ ) agonists significantly

reduced liver TAFI synthesis and ameliorated PH in mice and rats.<sup>93</sup> Thus, aTAFI is a novel and realistic therapeutic target of CTEPH. In addition to TAFI in CTEPH, there are several reports on the coagulation/fibrinolysis system published in a recent issue of *ATVB*. Interestingly, Tanratana et al<sup>102</sup> reported the effect of elevated plasma factor IXa activity in premenopausal women on hormonal contraception. They showed that donors on hormonal contraception demonstrate marked elevations in plasma factor IXa activity. Additionally, elevated plasma factor IXa activity correlated with reduced levels of TFPI- $\alpha$  (tissue factor pathway inhibitor- $\alpha$ ) and protein S. Thus, they concluded that elevated plasma factor IXa levels represent a mechanism for hormone-induced systemic hypercoagulability.<sup>102</sup> Raffield et al<sup>103</sup> reported the role of D-dimer in blacks. They showed that higher D-dimer was confirmed as an independent risk marker for future CVD and total mortality in blacks. Second, both acquired and genetic factors contributed to D-dimer variation among blacks. Additionally, they identified genetic factors that may account for sex and ethnic differences in D-dimer. Specifically, the African ancestral sickle cell variant (HBB rs334) was associated with higher D-dimer. Finally, they showed that a sex-specific association of the *F3* gene locus was seen in women, but not in men, which might partly explain the higher D-dimer levels in women compared with men.<sup>103</sup> Zhu et al<sup>104</sup> reported a novel thrombotic function of a human single-nucleotide polymorphism in STXBP5 (syntaxin-binding protein 5). The major finding of their study is that a human mutation in STXBP5 linked to vWF levels and thrombosis causes a thrombotic phenotype in mice. They used *CRISPR/Cas9* gene editing to create a mouse model of the human genetic variation. Mice carrying the minor allele of human single-nucleotide polymorphism rs1039084 at an orthologous locus decreased vWF exocytosis, decreased thrombosis, increased bleeding, and decreased platelet secretion.<sup>104</sup>

### Biomarkers for Heart Failure

Important biomarkers for patients with heart failure (HF) have been elucidated, including BNP, NT-proBNP, and ANP (atrial natriuretic peptide).<sup>105,106</sup> However, difficulties in the diagnosis of patients with HF with preserved ejection fraction are still encountered. We reported that myocardial fibrosis, evaluated from biopsy samples, predicts long-term prognosis of HF patients.<sup>107</sup> Moreover, we found that Rho-kinase plays a crucial role in the process of cardiac fibrosis in HF patients.<sup>62,108</sup> Postcapillary PH is a disorder with elevated pulmonary arterial pressure and pulmonary vascular resistance in patients with severe HF. Here, we found that the extent of postcapillary PH is a prognostic factor in HF patients.<sup>109</sup> As mentioned before, CyPA and Bsg play crucial roles in PASMC proliferation and pulmonary vascular remodeling.<sup>23,51</sup> Thus, we performed analyses to confirm the role of CyPA and Bsg in cardiac function and HF.<sup>22,110</sup> Consistent with other types of CVD, CyPA and Bsg enhanced ROS production, fibrosis, and hypertrophy in the heart in response to angiotensin II<sup>22</sup> and pressure overload.<sup>110</sup> Because CyPA is secreted in response to Rho-kinase activation and generates a vicious cycle of ROS production,<sup>11,12</sup> circulating CyPA can be a potential biomarker for HF patients, especially those with cardiac fibrosis.

Indeed, activation of Rho-kinase enhances myosin light-chain phosphorylation, induces VSMC contraction, and promotes inflammation and fibrosis.<sup>7</sup> Importantly, excessive activation of RhoA and its downstream Rho-kinase negatively regulate NO production in endothelial cells.<sup>111</sup> Additionally, this system promotes the secretion of growth factors, inducing cell proliferation and migration in VSMCs.<sup>51</sup> Indeed, this system plays an important role in the development of cardiac hypertrophy,<sup>112</sup> fibrosis,<sup>113</sup> arrhythmogenic right ventricular cardiomyopathy,<sup>114</sup> and PH.<sup>60,61</sup> In contrast, a selective Rho-kinase inhibitor, fasudil, suppresses this system and reduces the secretion of CyPA.<sup>19</sup> Consistently, administration of fasudil ameliorated HF and PH in animal models.<sup>61,115</sup> Because CyPA has been identified as a ROS-responsive protein that is also secreted by cardiac fibroblasts,<sup>19</sup> secreted CyPA may promote the development of cardiac fibrosis and HF. Thus, it is interesting to evaluate plasma levels of CyPA in patients with HF with preserved ejection fraction. Moreover, it is also important to find agents that inhibit CyPA secretion or CyPA binding to its receptors.<sup>10</sup> Indeed, many vascular cells secrete CyPA in response to ROS production and Rho-kinase activation.<sup>111</sup> Extracellular CyPA behaves as a chemoattractant in cooperation with other cytokines and chemokines in inflammatory cells.<sup>17</sup> The elucidation of the extracellular CyPA receptors will contribute to the development of novel therapies for CVD. However, in contrast to evidence on the role of extracellular CyPA, we had limited information regarding its specific receptors.<sup>10</sup> Because CyPA can be bound weakly with a variety of proteins, it has been difficult to establish a specific receptor.<sup>10,11,30</sup> Among them, Bsg has been a candidate as an extracellular receptor for CyPA.<sup>10</sup> Bsg has been known as a receptor for multiple ligands, including malaria parasites, CyPA, and sBsg (soluble Bsg) itself.<sup>10,110,116</sup> Interestingly, we found that Bsg is strongly expressed in cardiac fibroblasts, secreted by mechanical stretch, and activates matrix metalloproteinases in cardiac tissues.<sup>110</sup> Importantly, serum levels of sBsg were significantly increased in patients with HF and predicted all-cause death and HF hospitalization.<sup>110</sup> Moreover, they had strong correlations with serum levels of IL-8, adiponectin, and CyPA, suggesting the importance of CyPA and sBsg as biomarkers in HF patients.<sup>110</sup> Further study with a large cohort will provide more information as to the potential of these biomarkers in HF patients. As interactions between CyPA and Bsg promote the development of HF, the discovery and screening of agents that inhibit the binding of CyPA to Bsg will provide a novel therapeutic approach for HF patients. Additionally, in a recent *ATVB* publication, Varbo et al<sup>117</sup> reported the relationship between nonfasting triglycerides, LDL cholesterol, and HF risk. In 2 prospective studies of 113 554 individuals from the general population of Denmark, a stepwise higher risk of HF for stepwise higher nonfasting triglyceride level was shown. However, there was no association between LDL cholesterol and risk of HF. The mechanism of the association of higher nonfasting triglyceride level and higher risk of HF could be explained by the presence of triglyceride-rich lipoproteins, also known as remnants, and that remnant cholesterol has been shown to be associated with risk of ischemic heart disease, myocardial infarction, and all-cause mortality. Additionally, another explanation could be

triglyceride accumulation in heart muscle tissue, which, in obese and diabetic individuals, has been shown to cause lipotoxic cardiomyopathy and cardiac steatosis.<sup>117</sup>

### Conclusions

In this issue of Recent Highlights of *ATVB*, we reviewed >50 articles on the recent development of biomarkers in CVD. Although many points still remain to be clarified, recent basic research elucidated the precise mechanisms for the development of CVD. On the basis of this scientific progress, techniques for the early diagnosis, prognosis prediction, and evaluation of therapeutic effects should be developed. Now, translational research has become increasingly important. Even in basic research with animal models, it is important to confirm the clinical significance using human-derived pathological specimens to develop diagnostic tools and therapies. Thus, the organization of research environments that integrate broad areas from basic to clinical research becomes increasingly important.

### Acknowledgments

This work was supported in part by the grants-in-aid for Scientific Research (15H02535, 15H04816, and 15K15046) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan; the Ministry of Health, Labour, and Welfare, Tokyo, Japan (10102895); and the Japan Agency for Medical Research and Development, Tokyo, Japan (15ak0101035h0001, 16ek0109176h0001, and 17ek0109227h0001).

### Disclosures

None.

### References

- Shimokawa H. 2014 Williams Harvey Lecture: importance of coronary vasomotion abnormalities—from bench to bedside. *Eur Heart J*. 2014;35:3180–3193. doi: 10.1093/eurheartj/ehu427.
- Sato K. AMPK $\alpha$ 2 regulates hypoxia-inducible factor-1 $\alpha$  stability and neutrophil survival to promote vascular repair after ischemia. *Circ Res*. 2017;120:8–10. doi: 10.1161/CIRCRESAHA.116.310217.
- Shimokawa H, Sato K. Vascular function. *Arterioscler Thromb Vasc Biol*. 2014;34:2359–2362. doi: 10.1161/ATVBAHA.114.304119.
- Shimokawa H, Sunamura S, Sato K. RhoA/Rho-kinase in the cardiovascular system. *Circ Res*. 2016;118:352–366. doi: 10.1161/CIRCRESAHA.115.306532.
- Sato K, Kikuchi N, Kurosawa R, Shimokawa H. PDE1C negatively regulates growth factor receptor degradation and promotes VSMC proliferation. *Circ Res*. 2015;116:1098–1100. doi: 10.1161/CIRCRESAHA.115.306139.
- Sato K. Globotriaosylceramide induces endothelial dysfunction in Fabry disease. *Arterioscler Thromb Vasc Biol*. 2014;34:2–4. doi: 10.1161/ATVBAHA.113.302744.
- Sato K, Godo S, Saito H, Enkhjargal B, Shimokawa H. Dual roles of vascular-derived reactive oxygen species—with a special reference to hydrogen peroxide and cyclophilin A. *J Mol Cell Cardiol*. 2014;73:50–56. doi: 10.1016/j.yjmcc.2013.12.022.
- Sato K, Fukumoto Y, Sugimura K, Miura Y, Aoki T, et al. Plasma cyclophilin A is a novel biomarker for coronary artery disease. *Circ J*. 2013;77:447–455. doi: 10.1253/circj.CJ-12-0805.
- Sato K, Berk BC. Circulating smooth muscle progenitor cells: novel players in plaque stability. *Cardiovasc Res*. 2008;77:445–447. doi: 10.1093/cvr/cvm088.
- Sato K. Cyclophilin A in cardiovascular homeostasis and diseases. *Tohoku J Exp Med*. 2015;235:1–15. doi: 10.1620/tjem.235.1.
- Sato K, Nigro P, Berk BC. Oxidative stress and vascular smooth muscle cell growth: a mechanistic linkage by cyclophilin A. *Antioxid Redox Signal*. 2010;12:675–682. doi: 10.1089/ars.2009.2875.
- Sato K, Shimokawa H, Berk BC. Cyclophilin A: promising new target in cardiovascular therapy. *Circ J*. 2010;74:2249–2256.
- Galley JC, Straub AC. Redox control of vascular function. *Arterioscler Thromb Vasc Biol*. 2017;37:e178–e184. doi: 10.1161/ATVBAHA.117.309945.
- Sato K, Kagaya Y, Nakano M, et al. Important role of endogenous erythropoietin system in recruitment of endothelial progenitor cells in hypoxia-induced pulmonary hypertension in mice. *Circulation*. 2006;113:1442–1450. doi: 10.1161/CIRCULATIONAHA.105.583732.
- Tada H, Kagaya Y, Takeda M, Ohta J, Asaumi Y, Sato K, Ito K, Karibe A, Shirato K, Minegishi N, Shimokawa H. Endogenous erythropoietin system in non-hematopoietic lineage cells plays a protective role in myocardial ischemia/reperfusion. *Cardiovasc Res*. 2006;71:466–477. doi: 10.1016/j.cardiores.2006.05.010.
- Nakano M, Sato K, Fukumoto Y, Ito Y, Kagaya Y, Ishii N, Sugamura K, Shimokawa H. Important role of erythropoietin receptor to promote VEGF expression and angiogenesis in peripheral ischemia in mice. *Circ Res*. 2007;100:662–669. doi: 10.1161/01.RES.0000260179.43672.fe.
- Sato K, Matoba T, Suzuki J, O'Dell MR, Nigro P, Cui Z, Mohan A, Pan S, Li L, Jin ZG, Yan C, Abe J, Berk BC. Cyclophilin A mediates vascular remodeling by promoting inflammation and vascular smooth muscle cell proliferation. *Circulation*. 2008;117:3088–3098. doi: 10.1161/CIRCULATIONAHA.107.756106.
- Sato K, Fukumoto Y, Nakano M, Sugimura K, Nawata J, Demachi J, Karibe A, Kagaya Y, Ishii N, Sugamura K, Shimokawa H. Statin ameliorates hypoxia-induced pulmonary hypertension associated with down-regulated stromal cell-derived factor-1. *Cardiovasc Res*. 2009;81:226–234. doi: 10.1093/cvr/cvn244.
- Sato K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X, Mohan A, Yan C, Abe J, Illig KA, Berk BC. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. *Nat Med*. 2009;15:649–656. doi: 10.1038/nm.1958.
- Nigro P, Abe J, Woo CH, Sato K, McClain C, O'Dell MR, Lee H, Lim JH, Li JD, Heo KS, Fujiwara K, Berk BC. PKC $\zeta$  decreases eNOS protein stability via inhibitory phosphorylation of ERK5. *Blood*. 2010;116:1971–1979. doi: 10.1182/blood-2010-02-269134.
- Nigro P, Sato K, O'Dell MR, Soe NN, Cui Z, Mohan A, Abe J, Alexis JD, Sparks JD, Berk BC. Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med*. 2011;208:53–66. doi: 10.1084/jem.20101174.
- Sato K, Nigro P, Zeidan A, Soe NN, Jaffré F, Oikawa M, O'Dell MR, Cui Z, Menon P, Lu Y, Mohan A, Yan C, Blaxall BC, Berk BC. Cyclophilin A promotes cardiac hypertrophy in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2011;31:1116–1123. doi: 10.1161/ATVBAHA.110.214601.
- Sato K, Sato T, Kikuchi N, et al. Basigin mediates pulmonary hypertension by promoting inflammation and vascular smooth muscle cell proliferation. *Circ Res*. 2014;115:738–750. doi: 10.1161/CIRCRESAHA.115.304563.
- Ohtsuki T, Sato K, Omura J, Kikuchi N, Sato T, Kurosawa R, Nogi M, Sunamura S, Yaoita N, Aoki T, Tatebe S, Sugimura K, Takahashi J, Miyata S, Shimokawa H. Prognostic impacts of plasma levels of cyclophilin A in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2017;37:685–693. doi: 10.1161/ATVBAHA.116.308986.
- Li W, Dorans KS, Wilker EH, Rice MB, Ljungman PL, Schwartz JD, Coull BA, Koutrakis P, Gold DR, Keaney JF Jr, Vasan RS, Benjamin EJ, Mittleman MA. Short-term exposure to ambient air pollution and biomarkers of systemic inflammation: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol*. 2017;37:1793–1800. doi: 10.1161/ATVBAHA.117.309799.
- Bell G, Mora S, Greenland P, Tsai M, Gill E, Kaufman JD. Association of air pollution exposures with high-density lipoprotein cholesterol and particle number: the Multi-Ethnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2017;37:976–982. doi: 10.1161/ATVBAHA.116.308193.
- Sato K. Linoleic acid. A novel mechanism of endothelial cell dysfunction. *Circ J*. 2013;77:2702–2703.
- Sato K. Dipeptidyl peptidase-4 inhibitors: emerging player for vascular protection. *Circ J*. 2013;77:1156–1157. doi: 10.1253/circj.CJ-13-0064.
- Enkhjargal B, Godo S, Sawada A, Suvd N, Saito H, Noda K, Sato K, Shimokawa H. Endothelial AMP-activated protein kinase regulates blood pressure and coronary flow responses through hyperpolarization mechanism in mice. *Arterioscler Thromb Vasc Biol*. 2014;34:1505–1513. doi: 10.1161/ATVBAHA.114.303735.
- Sato K, Berk BC, Shimokawa H. Vascular-derived reactive oxygen species for homeostasis and diseases. *Nitric Oxide*. 2011;25:211–215. doi: 10.1016/j.niox.2011.04.005.

31. Chen X, Zhang Y, Chen Q, Li Q, Li Y, Ling W. Lower plasma fetuin-A levels are associated with a higher mortality risk in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2017;37:2213–2219. doi: 10.1161/ATVBAHA.117.309700.
32. Saita E, Miura K, Suzuki-Sugihara N, Miyata K, Ikemura N, Ohmori R, Ikegami Y, Kishimoto Y, Kondo K, Momiyama Y. Plasma soluble endoglin levels are inversely associated with the severity of coronary atherosclerosis—brief report. *Arterioscler Thromb Vasc Biol*. 2017;37:49–52. doi: 10.1161/ATVBAHA.116.308494.
33. Caselli C, Prontera C, Liga R, et al. Effect of coronary atherosclerosis and myocardial ischemia on plasma levels of high-sensitivity troponin T and NT-proBNP in patients with stable angina. *Arterioscler Thromb Vasc Biol*. 2016;36:757–764. doi: 10.1161/ATVBAHA.115.306818.
34. Gill D, Del Greco MF, Walker AP, Srai SKS, Laffan MA, Minelli C. The effect of iron status on risk of coronary artery disease: a Mendelian randomization study—brief report. *Arterioscler Thromb Vasc Biol*. 2017;37:1788–1792. doi: 10.1161/ATVBAHA.117.309757.
35. Doi T, Kataoka Y, Noguchi T, et al. Coronary artery ectasia predicts future cardiac events in patients with acute myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2017;37:2350–2355. doi: 10.1161/ATVBAHA.117.309683.
36. Kikuchi Y, Yasuda S, Aizawa K, Tsuburaya R, Ito Y, Takeda M, Nakayama M, Ito K, Takahashi J, Shimokawa H. Enhanced Rho-kinase activity in circulating neutrophils of patients with vasospastic angina: a possible biomarker for diagnosis and disease activity assessment. *J Am Coll Cardiol*. 2011;58:1231–1237. doi: 10.1016/j.jacc.2011.05.046.
37. Nihei T, Takahashi J, Hao K, Kikuchi Y, Odaka Y, Tsuburaya R, Nishimiya K, Matsumoto Y, Ito K, Miyata S, Sakata Y, Shimokawa H. Prognostic impacts of Rho-kinase activity in circulating leucocytes in patients with vasospastic angina [published online ahead of print November 20, 2017]. *Eur Heart J*. doi: 10.1093/eurheartj/ehx657.
38. Ohyama K, Matsumoto Y, Takanami K, et al. Coronary adventitial and perivascular adipose tissue inflammation in patients with vasospastic angina. *J Am Coll Cardiol*. 2018;71:414–425. doi: 10.1016/j.jacc.2017.11.046.
39. Odaka Y, Takahashi J, Tsuburaya R, Nishimiya K, Hao K, Matsumoto Y, Ito K, Sakata Y, Miyata S, Manita D, Hirawatari Y, Shimokawa H. Plasma concentration of serotonin is a novel biomarker for coronary microvascular dysfunction in patients with suspected angina and unobstructive coronary arteries. *Eur Heart J*. 2017;38:489–496. doi: 10.1093/eurheartj/ehw448.
40. Takahashi K, Matsumoto Y, Do e Z, Kanazawa M, Satoh K, Shimizu T, Sato A, Fukumoto Y, Shimokawa H. Combination therapy with atorvastatin and amlodipine suppresses angiotensin II-induced aortic aneurysm formation. *PLoS One*. 2013;8:e72558. doi: 10.1371/journal.pone.0072558.
41. Leung LY, Bartz TM, Rice K, Floyd J, Psaty B, Gutierrez J, Longstreth WT Jr, Mukamal KJ. Blood pressure and heart rate measures associated with increased risk of covert brain infarction and worsening leukoaraiosis in older adults. *Arterioscler Thromb Vasc Biol*. 2017;37:1579–1586. doi: 10.1161/ATVBAHA.117.309298.
42. Lin X, Racette SB, Ma L, Wallendorf M, Dávila-Román VG, Ostlund RE Jr. Endogenous cholesterol excretion is negatively associated with carotid intima-media thickness in humans. *Arterioscler Thromb Vasc Biol*. 2017;37:2364–2369. doi: 10.1161/ATVBAHA.117.310081.
43. Nguyen MA, Karunakaran D, Geoffrion M, Cheng HS, Tandoc K, Perisic Matic L, Hedin U, Maegdefessel L, Fish JE, Rayner KJ. Extracellular vesicles secreted by atherogenic macrophages transfer MicroRNA to inhibit cell migration. *Arterioscler Thromb Vasc Biol*. 2018;38:49–63. doi: 10.1161/ATVBAHA.117.309795.
44. Heffron SP, Rockman CB, Adelman MA, Gianos E, Guo Y, Xu JF, Berger JS. Greater frequency of fruit and vegetable consumption is associated with lower prevalence of peripheral artery disease. *Arterioscler Thromb Vasc Biol*. 2017;37:1234–1240. doi: 10.1161/ATVBAHA.116.308474.
45. Goettsch C, Iwata H, Hutcheson JD, O'Donnell CJ, Chapurlat R, Cook NR, Aikawa M, Szulc P, Aikawa E. Serum sortilin associates with aortic calcification and cardiovascular risk in men. *Arterioscler Thromb Vasc Biol*. 2017;37:1005–1011. doi: 10.1161/ATVBAHA.116.308932.
46. He YH, Wang XQ, Zhang J, Liu ZH, Pan WQ, Shen Y, Zhu ZB, Wang LJ, Yan XX, Yang K, Zhang RY, Shen WF, Ding FH, Lu L. Association of serum HMGB2 levels with in-stent restenosis: HMGB2 promotes neointimal hyperplasia in mice with femoral artery injury and proliferation and migration of VSMCs. *Arterioscler Thromb Vasc Biol*. 2017;37:717–729. doi: 10.1161/ATVBAHA.116.308210.
47. Small A, Kiss D, Giri J, Anwaruddin S, Siddiqi H, Guerraty M, Chirinos JA, Ferrari G, Rader DJ. Biomarkers of calcific aortic valve disease. *Arterioscler Thromb Vasc Biol*. 2017;37:623–632. doi: 10.1161/ATVBAHA.116.308615.
48. Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, Salomaa V, Laaksonen R. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. *Arterioscler Thromb Vasc Biol*. 2016;36:2424–2430. doi: 10.1161/ATVBAHA.116.307497.
49. Amor AJ, Ortega E, Perea V, Cofán M, Sala-Vila A, Nuñez I, Gilabert R, Ros E. Relationship between total serum bilirubin levels and carotid and femoral atherosclerosis in familial dyslipidemia. *Arterioscler Thromb Vasc Biol*. 2017;37:2356–2363. doi: 10.1161/ATVBAHA.117.310071.
50. Prats-Puig A, Moreno M, Carreras-Badosa G, Bassols J, Ricart W, López-Bermejo A, Fernández-Real JM. Serum ferritin relates to carotid intima-media thickness in offspring of fathers with higher serum ferritin levels. *Arterioscler Thromb Vasc Biol*. 2016;36:174–180. doi: 10.1161/ATVBAHA.115.306396.
51. Yaoita N, Satoh K, Shimokawa H. Novel therapeutic targets of pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2016;36:e97–e102. doi: 10.1161/ATVBAHA.116.308263.
52. Omura J, Satoh K, Kikuchi N, et al. Protective roles of endothelial AMP-activated protein kinase against hypoxia-induced pulmonary hypertension in mice. *Circ Res*. 2016;119:197–209. doi: 10.1161/CIRCRESAHA.115.308178.
53. Satoh K, Fukumoto Y, Nakano M, Kagaya Y, Shimokawa H. Emergence of the erythropoietin/erythropoietin receptor system as a novel cardiovascular therapeutic target. *J Cardiovasc Pharmacol*. 2011;58:570–574. doi: 10.1097/FJC.0b013e318235e7bb.
54. Kagaya Y, Asaumi Y, Wang W, Takeda M, Nakano M, Satoh K, Fukumoto Y, Shimokawa H. Current perspectives on protective roles of erythropoietin in cardiovascular system: erythropoietin receptor as a novel therapeutic target. *Tohoku J Exp Med*. 2012;227:83–91.
55. Ikarashi N, Toba K, Kato K, et al. Erythropoietin, but not asialoerythropoietin or carbamyl-erythropoietin, attenuates monocrotaline-induced pulmonary hypertension in rats. *Clin Exp Hypertens*. 2012;34:575–581. doi: 10.3109/10641963.2012.681728.
56. Little JA, McGowan VR, Kato GJ, Partovi KS, Feld JJ, Maric I, Martyr S, Taylor JG 6th, Machado RF, Heller T, Castro O, Gladwin MT. Combination erythropoietin-hydroxyurea therapy in sickle cell disease: experience from the National Institutes of Health and a literature review. *Haematologica*. 2006;91:1076–1083.
57. Swedberg K, Young JB, Anand IS, Cheng S, Desai AS, Diaz R, Maggioni AP, McMurray JJ, O'Connor C, Pfeffer MA, Solomon SD, Sun Y, Tendera M, van Veldhuisen DJ; RED-HF Committees; RED-HF Investigators. Treatment of anemia with darbepoetin alfa in systolic heart failure. *N Engl J Med*. 2013;368:1210–1219. doi: 10.1056/NEJMoa1214865.
58. Shimokawa H, Satoh K. Light and dark of reactive oxygen species for vascular function: 2014 ASVB (Asian Society of Vascular Biology). *J Cardiovasc Pharmacol*. 2015;65:412–418. doi: 10.1097/FJC.0000000000000159.
59. Rabiyousefi M, Soroosh P, Satoh K, Date F, Ishii N, Yamashita M, Oka M, McMurtry IF, Shimokawa H, Nose M, Sugamura K, Ono M. Indispensable roles of OX40L-derived signal and epistatic genetic effect in immune-mediated pathogenesis of spontaneous pulmonary hypertension. *BMC Immunol*. 2011;12:67. doi: 10.1186/1471-2172-12-67.
60. Shimizu T, Fukumoto Y, Tanaka S, Satoh K, Ikeda S, Shimokawa H. Crucial role of ROCK2 in vascular smooth muscle cells for hypoxia-induced pulmonary hypertension in mice. *Arterioscler Thromb Vasc Biol*. 2013;33:2780–2791. doi: 10.1161/ATVBAHA.113.301357.
61. Elias-Al-Mamun M, Satoh K, Tanaka S, Shimizu T, Nergui S, Miyata S, Fukumoto Y, Shimokawa H. Combination therapy with fasudil and sildenafl ameliorates monocrotaline-induced pulmonary hypertension and survival in rats. *Circ J*. 2014;78:967–976. doi: 10.1253/circj.CJ-13-1174.
62. Doe Z, Fukumoto Y, Sugimura K, et al. Rho-kinase activation in patients with heart failure. *Circ J*. 2013;77:2542–2550. doi: 10.1253/circj.CJ-13-0397.
63. Karoor V, Fini MA, Loomis Z, Sullivan T, Hersh LB, Gerasimovskaya E, Irwin D, Dempsey EC. Sustained activation of Rho GTPases promotes a synthetic pulmonary artery smooth muscle cell phenotype in neprilysin null mice. *Arterioscler Thromb Vasc Biol*. 2018;38:154–163. doi: 10.1161/ATVBAHA.117.310207.
64. Xue C, Sowden M, Berk BC. Extracellular cyclophilin A, especially acetylated, causes pulmonary hypertension by stimulating endothelial apoptosis, redox stress, and inflammation. *Arterioscler Thromb Vasc Biol*. 2017;37:1138–1146. doi: 10.1161/ATVBAHA.117.309212.
65. Jiang L, Konishi H, Nurwidya F, Satoh K, Takahashi F, Ebinuma H, Fujimura K, Takasu K, Jiang M, Shimokawa H, Bujo H, Daida H. Deletion of LR11 attenuates hypoxia-induced pulmonary arterial smooth muscle



- cell proliferation with medial thickening in mice. *Arterioscler Thromb Vasc Biol.* 2016;36:1972–1979. doi: 10.1161/ATVBAHA.116.307900.
66. Miura Y, Fukumoto Y, Sugimura K, Oikawa M, Nakano M, Tatebe S, Miyamichi S, Satoh K, Shimokawa H. Identification of new prognostic factors of pulmonary hypertension. *Circ J.* 2010;74:1965–1971. doi: 10.1253/circj.CJ-10-0270.
  67. Tatebe S, Fukumoto Y, Oikawa-Wakayama M, Sugimura K, Satoh K, Miura Y, Aoki T, Nochioka K, Miura M, Yamamoto S, Tashiro M, Kagaya Y, Shimokawa H. Enhanced [18F]fluorodeoxyglucose accumulation in the right ventricular free wall predicts long-term prognosis of patients with pulmonary hypertension: a preliminary observational study. *Eur Heart J Cardiovasc Imaging.* 2014;15:666–672. doi: 10.1093/ehjci/et276.
  68. Dai Z, Sugimura K, Fukumoto Y, Tatebe S, Miura Y, Nochioka K, Aoki T, Miyamichi-Yamamoto S, Yaoita N, Satoh K, Shimokawa H. Visualization of complete regression of pulmonary arterial remodeling on optical coherence tomography in a patient with pulmonary arterial hypertension. *Circ J.* 2014;78:2771–2773.
  69. Dai Z, Fukumoto Y, Tatebe S, Sugimura K, Miura Y, Nochioka K, Aoki T, Miyamichi-Yamamoto S, Yaoita N, Satoh K, Shimokawa H. OCT imaging for the management of pulmonary hypertension. *JACC Cardiovasc Imaging.* 2014;7:843–845. doi: 10.1016/j.jcmg.2014.01.020.
  70. Meloche J, Lampron MC, Nadeau V, Maltais M, Potus F, Lambert C, Tremblay E, Vitry G, Breuils-Bonnet S, Bouché O, Charbonneau E, Provencher S, Paulin R, Bonnet S. Implication of inflammation and epigenetic readers in coronary artery remodeling in patients with pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol.* 2017;37:1513–1523. doi: 10.1161/ATVBAHA.117.309156.
  71. Sonneveld MA, Franco OH, Ikram MA, Hofman A, Kavousi M, de Maat MP, Leebeek FW, Von Willebrand factor, ADAMTS13, and the risk of mortality: the Rotterdam study. *Arterioscler Thromb Vasc Biol.* 2016;36:2446–2451. doi: 10.1161/ATVBAHA.116.308225.
  72. Song SH, Kim K, Jo EK, Kim YW, Kwon JS, Bae SS, Sung JH, Park SG, Kim JT, Suh W. Fibroblast growth factor 12 is a novel regulator of vascular smooth muscle cell plasticity and fate. *Arterioscler Thromb Vasc Biol.* 2016;36:1928–1936. doi: 10.1161/ATVBAHA.116.308017.
  73. Hata J, Mukai N, Nagata M, Ohara T, Yoshida D, Kishimoto H, Shibata M, Hirakawa Y, Endo M, Ago T, Kitazono T, Oike Y, Kiyohara Y, Ninomiya T. Serum angiopoietin-like protein 2 is a novel risk factor for cardiovascular disease in the community: the Hisayama study. *Arterioscler Thromb Vasc Biol.* 2016;36:1686–1691. doi: 10.1161/ATVBAHA.116.307291.
  74. Januzzi JL Jr, Lyass A, Liu Y, Gaggin H, Trebnick A, Maisel AS, D'Agostino RB Sr, Wang TJ, Massaro J, Vasan RS. Circulating proneurotensin concentrations and cardiovascular disease events in the community: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol.* 2016;36:1692–1697. doi: 10.1161/ATVBAHA.116.307847.
  75. Herder C, de Las Heras Gala T, Carstensen-Kirberg M, et al. Circulating levels of interleukin 1-receptor antagonist and risk of cardiovascular disease: meta-analysis of six population-based cohorts. *Arterioscler Thromb Vasc Biol.* 2017;37:1222–1227. doi: 10.1161/ATVBAHA.117.309307.
  76. Spiga R, Marini MA, Mancuso E, Di Fatta C, Fuoco A, Perticone F, Andreozzi F, Mannino GC, Sesti G. Uric acid is associated with inflammatory biomarkers and induces inflammation via activating the NF- $\kappa$ B signaling pathway in HepG2 cells. *Arterioscler Thromb Vasc Biol.* 2017;37:1241–1249. doi: 10.1161/ATVBAHA.117.309128.
  77. Liu G, Ding M, Chiuve SE, Rimm EB, Franks PW, Meigs JB, Hu FB, Sun Q. Plasma levels of fatty acid-binding protein 4, retinol-binding protein 4, high-molecular-weight adiponectin, and cardiovascular mortality among men with type 2 diabetes: a 22-year prospective study. *Arterioscler Thromb Vasc Biol.* 2016;36:2259–2267. doi: 10.1161/ATVBAHA.116.308320.
  78. Xue L, Borné Y, Mattisson IY, Wigren M, Melander O, Ohro-Melander M, Bengtsson E, Fredrikson GN, Nilsson J, Engström G. FADD, caspase-3, and caspase-8 and incidence of coronary events. *Arterioscler Thromb Vasc Biol.* 2017;37:983–989. doi: 10.1161/ATVBAHA.117.308995.
  79. Moriarty PM, Varvel SA, Gordts PL, McConnell JP, Tsimikas S. Lipoprotein(a) mass levels increase significantly according to APOE genotype: an analysis of 431 239 patients. *Arterioscler Thromb Vasc Biol.* 2017;37:580–588. doi: 10.1161/ATVBAHA.116.308704.
  80. Bajaj A, Damrauer SM, Anderson AH, et al; Chronic Renal Insufficiency Cohort (CRIC) Study Investigators\*. Lipoprotein(a) and risk of myocardial infarction and death in chronic kidney disease: findings from the CRIC study (Chronic Renal Insufficiency Cohort). *Arterioscler Thromb Vasc Biol.* 2017;37:1971–1978. doi: 10.1161/ATVBAHA.117.309920.
  81. Kim MK, Han K, Koh ES, Kim HS, Kwon HS, Park YM, Yoon KH, Lee SH. Variability in total cholesterol is associated with the risk of end-stage renal disease: a nationwide population-based study. *Arterioscler Thromb Vasc Biol.* 2017;37:1963–1970. doi: 10.1161/ATVBAHA.117.309803.
  82. Rydén M, Arner P. Subcutaneous adipocyte lipolysis contributes to circulating lipid levels. *Arterioscler Thromb Vasc Biol.* 2017;37:1782–1787. doi: 10.1161/ATVBAHA.117.309759.
  83. Prasad A, Clopton P, Ayers C, Khera A, de Lemos JA, Witztum JL, Tsimikas S. Relationship of autoantibodies to MDA-LDL and ApoB-immune complexes to sex, ethnicity, subclinical atherosclerosis, and cardiovascular events. *Arterioscler Thromb Vasc Biol.* 2017;37:1213–1221. doi: 10.1161/ATVBAHA.117.309101.
  84. Björkbacka H, Alm R, Persson M, Hedblad B, Nilsson J, Fredrikson GN. Low levels of apolipoprotein B-100 autoantibodies are associated with increased risk of coronary events. *Arterioscler Thromb Vasc Biol.* 2016;36:765–771. doi: 10.1161/ATVBAHA.115.306938.
  85. Antiochos P, Marques-Vidal P, Virzi J, Pagano S, Satta N, Hartley O, Montecucco F, Mach F, Kutalik Z, Waeber G, Vollenweider P, Vuilleumier N. Impact of CD14 polymorphisms on anti-apolipoprotein A-I IgG-related coronary artery disease prediction in the general population. *Arterioscler Thromb Vasc Biol.* 2017;37:2342–2349. doi: 10.1161/ATVBAHA.117.309602.
  86. van Capelleveen JC, Bernelot Moens SJ, Yang X, Kastelein JJP, Wareham NJ, Zwiderman AH, Stroes ESG, Witztum JL, Hovingh GK, Khaw KT, Boekholdt SM, Tsimikas S. Apolipoprotein C-III levels and incident coronary artery disease risk: the EPIC-Norfolk prospective population study. *Arterioscler Thromb Vasc Biol.* 2017;37:1206–1212. doi: 10.1161/ATVBAHA.117.309007.
  87. Frej C, Mendez AJ, Ruiz M, Castillo M, Hughes TA, Dahlbäck B, Goldberg RB. A shift in ApoM/S1P between HDL-particles in women with type 1 diabetes mellitus is associated with impaired anti-inflammatory effects of the ApoM/S1P complex. *Arterioscler Thromb Vasc Biol.* 2017;37:1194–1205. doi: 10.1161/ATVBAHA.117.309275.
  88. Bernelot Moens SJ, Verweij SL, Schnitzler JG, Stiekema LCA, Bos M, Langsted A, Kuijk C, Bekkering S, Voermans C, Verberne HJ, Nordestgaard BG, Stroes ESG, Kroon J. Remnant cholesterol elicits arterial wall inflammation and a multilevel cellular immune response in humans. *Arterioscler Thromb Vasc Biol.* 2017;37:969–975. doi: 10.1161/ATVBAHA.116.308834.
  89. Puri R, Nissen SE, Shao M, Elshazly MB, Kataoka Y, Kapadia SR, Tuzcu EM, Nicholls SJ. Non-HDL cholesterol and triglycerides: implications for coronary atheroma progression and clinical events. *Arterioscler Thromb Vasc Biol.* 2016;36:2220–2228. doi: 10.1161/ATVBAHA.116.307601.
  90. Kockx M, Glaros E, Leung B, Ng TW, Berbée JF, Deswaerte V, Nawara D, Quinn C, Rye KA, Jessup W, Rensen PC, Meikle PJ, Kritharides L. Low-density lipoprotein receptor-dependent and low-density lipoprotein receptor-independent mechanisms of cyclosporin A-induced dyslipidemia. *Arterioscler Thromb Vasc Biol.* 2016;36:1338–1349. doi: 10.1161/ATVBAHA.115.307030.
  91. Ogura M, Hori M, Harada-Shiba M. Association between cholesterol efflux capacity and atherosclerotic cardiovascular disease in patients with familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2016;36:181–188. doi: 10.1161/ATVBAHA.115.306665.
  92. Yaoita N, Satoh K, Satoh T, Sugimura K, Tatebe S, Yamamoto S, Aoki T, Miura M, Miyata S, Kawamura T, Horiuchi H, Fukumoto Y, Shimokawa H. Thrombin-activatable fibrinolysis inhibitor in chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol.* 2016;36:1293–1301. doi: 10.1161/ATVBAHA.115.306845.
  93. Satoh T, Satoh K, Yaoita N, Kikuchi N, Omura J, Kurosawa R, Numano K, Al-Mamun E, Siddique MA, Sunamura S, Nogi M, Suzuki K, Miyata S, Morser J, Shimokawa H. Activated TAFI promotes the development of chronic thromboembolic pulmonary hypertension: a possible novel therapeutic target. *Circ Res.* 2017;120:1246–1262. doi: 10.1161/CIRCRESAHA.117.310640.
  94. Tatebe S, Fukumoto Y, Sugimura K, Nakano M, Miyamichi S, Satoh K, Oikawa M, Shimokawa H. Optical coherence tomography as a novel diagnostic tool for distal type chronic thromboembolic pulmonary hypertension. *Circ J.* 2010;74:1742–1744.
  95. Sugimura K, Fukumoto Y, Satoh K, Nochioka K, Miura Y, Aoki T, Tatebe S, Miyamichi-Yamamoto S, Shimokawa H. Percutaneous transluminal pulmonary angioplasty markedly improves pulmonary hemodynamics and long-term prognosis in patients with chronic thromboembolic pulmonary hypertension. *Circ J.* 2012;76:485–488.
  96. Tatebe S, Fukumoto Y, Sugimura K, Miura Y, Nochioka K, Aoki T, Miura M, Yamamoto S, Yaoita N, Satoh K, Shimokawa H. Optical coherence tomography is superior to intravascular ultrasound for diagnosis of distal-type chronic thromboembolic pulmonary hypertension. *Circ J.* 2013;77:1081–1083.

97. Aoki T, Sugimura K, Tatebe S, Miura M, Yamamoto S, Yaoita N, Suzuki H, Sato H, Kozu K, Konno R, Miyata S, Nochioka K, Satoh K, Shimokawa H. Comprehensive evaluation of the effectiveness and safety of balloon pulmonary angioplasty for inoperable chronic thrombo-embolic pulmonary hypertension: long-term effects and procedure-related complications. *Eur Heart J*. 2017;38:3152–3159. doi: 10.1093/eurheartj/ehx530.
98. Sugimura K, Fukumoto Y, Miura Y, Nochioka K, Miura M, Tatebe S, Aoki T, Satoh K, Yamamoto S, Yaoita N, Shimokawa H. Three-dimensional-optical coherence tomography imaging of chronic thromboembolic pulmonary hypertension. *Eur Heart J*. 2013;34:2121. doi: 10.1093/eurheartj/ehs203.
99. Aoki T, Sugimura K, Nochioka K, Miura M, Tatebe S, Yamamoto S, Yaoita N, Suzuki H, Sato H, Kozu K, Miyata S, Satoh K, Shimokawa H. Effects of balloon pulmonary angioplasty on oxygenation in patients with chronic thromboembolic pulmonary hypertension - importance of intrapulmonary shunt. *Circ J*. 2016;80:2227–2234. doi: 10.1253/circj.CJ-16-0254.
100. Sato H, Ota H, Sugimura K, Aoki T, Tatebe S, Miura M, Yamamoto S, Yaoita N, Suzuki H, Satoh K, Takase K, Shimokawa H. Balloon pulmonary angioplasty improves biventricular functions and pulmonary flow in chronic thromboembolic pulmonary hypertension. *Circ J*. 2016;80:1470–1477. doi: 10.1253/circj.CJ-15-1187.
101. Tatebe S, Sugimura K, Aoki T, Miura M, Nochioka K, Yaoita N, Suzuki H, Sato H, Yamamoto S, Satoh K, Fukumoto Y, Shimokawa H. Multiple beneficial effects of balloon pulmonary angioplasty in patients with chronic thromboembolic pulmonary hypertension. *Circ J*. 2016;80:980–988. doi: 10.1253/circj.CJ-15-1212.
102. Tanratana P, Ellery P, Westmark P, Mast AE, Sheehan JP. Elevated plasma factor IXa activity in premenopausal women on hormonal contraception. *Arterioscler Thromb Vasc Biol*. 2018;38:266–274. doi: 10.1161/ATVBAHA.117.309919.
103. Raffield LM, Zakai NA, Duan Q, et al; NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, Hematology & Hemostasis TOPMed Working Group\*. D-Dimer in African Americans: whole genome sequence analysis and relationship to cardiovascular disease risk in the Jackson Heart Study. *Arterioscler Thromb Vasc Biol*. 2017;37:2220–2227. doi: 10.1161/ATVBAHA.117.310073.
104. Zhu QM, Ko KA, Ture S, Mastrangelo MA, Chen MH, Johnson AD, O'Donnell CJ, Morrell CN, Miano JM, Lowenstein CJ. Novel thrombotic function of a human SNP in STXBP5 revealed by CRISPR/Cas9 gene editing in mice. *Arterioscler Thromb Vasc Biol*. 2017;37:264–270. doi: 10.1161/ATVBAHA.116.308614.
105. Tokudome T, Kishimoto I, Yamahara K, Osaki T, Minamino N, Horio T, Sawai K, Kawano Y, Miyazato M, Sata M, Kohno M, Nakao K, Kangawa K. Impaired recovery of blood flow after hind-limb ischemia in mice lacking guanylyl cyclase-A, a receptor for atrial and brain natriuretic peptides. *Arterioscler Thromb Vasc Biol*. 2009;29:1516–1521. doi: 10.1161/ATVBAHA.109.187526.
106. Mutlu U, Ikram MA, Hofman A, de Jong PT, Klaver CC, Ikram MK. N-terminal Pro-B-type natriuretic peptide is related to retinal microvascular damage: the Rotterdam Study. *Arterioscler Thromb Vasc Biol*. 2016;36:1698–1702. doi: 10.1161/ATVBAHA.116.307545.
107. Aoki T, Fukumoto Y, Sugimura K, Oikawa M, Satoh K, Nakano M, Nakayama M, Shimokawa H. Prognostic impact of myocardial interstitial fibrosis in non-ischemic heart failure. Comparison between preserved and reduced ejection fraction heart failure. *Circ J*. 2011;75:2605–2613.
108. Abe Y, Ito K, Hao K, Shindo T, Ogata T, Kagaya Y, Kurosawa R, Nishimiya K, Satoh K, Miyata S, Kawakami K, Shimokawa H. Extracorporeal low-energy shock-wave therapy exerts anti-inflammatory effects in a rat model of acute myocardial infarction. *Circ J*. 2014;78:2915–2925.
109. Tatebe S, Fukumoto Y, Sugimura K, Miyamichi-Yamamoto S, Aoki T, Miura Y, Nochioka K, Satoh K, Shimokawa H. Clinical significance of reactive post-capillary pulmonary hypertension in patients with left heart disease. *Circ J*. 2012;76:1235–1244.
110. Suzuki K, Satoh K, Ikeda S, et al. Basigin promotes cardiac fibrosis and failure in response to chronic pressure overload in mice. *Arterioscler Thromb Vasc Biol*. 2016;36:636–646. doi: 10.1161/ATVBAHA.115.306686.
111. Satoh K, Fukumoto Y, Shimokawa H. Rho-kinase: important new therapeutic target in cardiovascular diseases. *Am J Physiol Heart Circ Physiol*. 2011;301:H287–H296. doi: 10.1152/ajpheart.00327.2011.
112. Ikeda S, Satoh K, Kikuchi N, Miyata S, Suzuki K, Omura J, Shimizu T, Kobayashi K, Kobayashi K, Fukumoto Y, Sakata Y, Shimokawa H. Crucial role of rho-kinase in pressure overload-induced right ventricular hypertrophy and dysfunction in mice. *Arterioscler Thromb Vasc Biol*. 2014;34:1260–1271. doi: 10.1161/ATVBAHA.114.303320.
113. Kudo S, Satoh K, Nogi M, Suzuki K, Sunamura S, Omura J, Kikuchi N, Kurosawa R, Satoh T, Minami T, Ikeda S, Miyata S, Shimokawa H. SmgGDS as a crucial mediator of the inhibitory effects of statins on cardiac hypertrophy and fibrosis: novel mechanism of the pleiotropic effects of statins. *Hypertension*. 2016;67:878–889. doi: 10.1161/HYPERTENSIONAHA.115.07089.
114. Ellawindy A, Satoh K, Sunamura S, et al. Rho-kinase inhibition during early cardiac development causes arrhythmogenic right ventricular cardiomyopathy in mice. *Arterioscler Thromb Vasc Biol*. 2015;35:2172–2184. doi: 10.1161/ATVBAHA.115.305872.
115. Fukui S, Fukumoto Y, Suzuki J, Saji K, Nawata J, Tawara S, Shinozaki T, Kagaya Y, Shimokawa H. Long-term inhibition of Rho-kinase ameliorates diastolic heart failure in hypertensive rats. *J Cardiovasc Pharmacol*. 2008;51:317–326. doi: 10.1097/FJC.0b013e31816533b7.
116. Kato N, Yuzawa Y, Kosugi T, Hobo A, Sato W, Miwa Y, Sakamoto K, Matsuo S, Kadomatsu K. The E-selectin ligand basigin/CD147 is responsible for neutrophil recruitment in renal ischemia/reperfusion. *J Am Soc Nephrol*. 2009;20:1565–1576. doi: 10.1681/ASN.2008090957.
117. Varbo A, Nordestgaard BG. Nonfasting triglycerides, low-density lipoprotein cholesterol, and heart failure risk: two cohort studies of 113 554 individuals. *Arterioscler Thromb Vasc Biol*. 2018;38:464–472. doi: 10.1161/ATVBAHA.117.310269.

KEY WORDS: air pollution ■ biomarkers ■ cardiovascular diseases ■ cyclophilin A ■ reactive oxygen species