Genetic Regulation of Endothelial Vasomotor Function

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The endothelium plays an important role in the regulation of vasomotor tone and the maintenance of vascular integrity. Endothelial dysfunction, i.e., impaired endothelial dependent dilation, is a fundamental component of the pathogenesis of cardiovascular disease. Although endothelial dysfunction is associated with a number of cardiovascular disease risk factors, those risk factors are not the only determinants of endothelial dysfunction. Despite knowing many molecules involved in endothelial signaling pathways, the genetic contribution to endothelial function has yet to be fully elucidated. This mini-review summarizes current evidence supporting the genetic contribution to endothelial vasomotor function. Findings from population-based studies, association studies for candidate genes, and unbiased large genomic scale studies in humans and rodent models are discussed. A brief synopsis of the current studies addressing the genetic regulation of endothelial responses to exercise training is also included.

Keywords: flow-mediated dilation, endothelium-dependent vasodilator, heritability, polymorphism, association studies, rodent strain comparison

INTRODUCTION

The endothelium is an important modulator of vascular function, sensing changes in hemodynamic forces and blood-borne signals, and responding by releasing vasoactive molecules (Hintze and Vatner, 1984; Pohl et al., 1986; Rubanyi et al., 1986; Sinoway et al., 1989; Koller and Kaley, 1991; Hecker et al., 1993). Clinically, endothelial dysfunction is characterized by a reduced response to infusion of endothelium-dependent vasodilators, such as acetylcholine (ACH; Nabel et al., 1990; Hasdai and Lerman, 1999), or impaired flow-mediated dilation (FMD). FMD measures vasodilation induced by reactive hyperemia after release of acute occlusion of the brachial artery (Celermajer et al., 1992). This acute increase in blood flow exerts shear forces on the vessel that stimulate endothelial cells to release primarily nitric oxide (NO) which subsequently relaxes vascular smooth muscle (Pohl et al., 1986; Rubanyi et al., 1986). Endothelial dysfunction is a predictor of future cardiovascular events (Yeboah et al., 2009; Inaba et al., 2010; Ras et al., 2013) and also contributes to the pathology of chronic diseases including diabetes (McVeigh et al., 1992; Williams et al., 1996), chronic kidney disease (Annuk et al., 2001; Stam et al., 2006), and Alzheimer's disease (Dede et al., 2007; Kelleher and Soiza, 2013).

Chan et al. estimated that known risk factors for coronary heart disease account for <20% of variation in vascular responses to vasodilator agents (Chan et al., 2001). Differences in resting blood flow may account for up to another 45%. Therefore, the remaining 30–40% of variation in vascular function is unexplained. Although one potential factor is genetic variation, genetic regulation of endothelial vasomotor function is poorly understood. This mini-review will concentrate on the role of genetic variants on vascular function, emphasizing endothelium-dependent responses.
We will summarize the results from candidate gene studies in humans and rodents and highlight genome-wide studies of genetic regulation of endothelial function.

GENETIC REGULATION OF ENDOTHELIAL FUNCTION IN HUMANS

Familial Resemblance and Heritability
In children and first-degree relatives of individuals with premature coronary disease, impaired endothelial function occurs before onset of overt disease and is significantly and independently correlated with family history of premature coronary disease (Clarkson et al., 1997; Gaeta et al., 2000). Furthermore, twin and family studies suggest endothelial function or FMD is a heritable trait. A significant familial aggregation was reported for FMD measured in 81 nuclear families with a heritability estimate of 0.58 for FMD in sibling pairs (Ryabikov et al., 2007). The Northern Manhattan Family Study reported heritability for FMD of 0.17 in Hispanic Caucasians after adjusting for age, sex, and cardiovascular disease (CVD) risk factors (Suzuki et al., 2008). Several twin studies have reported higher resemblance in FMD between monozygotic twins than dizygotic twins with heritability estimates between 0.24 and 0.44 (Jarti et al., 2002; Zhao et al., 2007; Hopkins et al., 2010). Two population-based studies estimated heritability for FMD to be 0.14–0.16 after accounting for confounding variables (Benjamin et al., 2004; Fisch et al., 2015). Collectively those findings support a role for genetics in development of endothelial dysfunction and heritability of endothelial function measured as FMD.

Candidate Gene Studies
To date, the majority of research focusing on the genetic basis for endothelial vasomotor function has used the candidate gene approach. Association studies in humans have shown polymorphisms in genes related to vascular function [e.g., angiotensin converting enzyme, angiotensin II type 1 receptor, cytochrome b-245 alpha chain (CYBA), nitric oxide synthase 3 (NOS3), and GTP cyclohydrolase 1 (GCH1)] exhibit a range of effects on endothelial function (Table 1; Celermajer et al., 1994; Schächinger et al., 2001; Rossi et al., 2003; Schneider et al., 2003; Cattaruzza et al., 2004; Fricker et al., 2004; Paradossi et al., 2004; Demirel et al., 2005; Fan et al., 2007; Kiliszek et al., 2007; Antoniades et al., 2008; Ingelsson et al., 2008; Liao et al., 2010; Akpinar et al., 2014; Dong et al., 2014; Rafiq et al., 2014; Wolkow et al., 2014; Li et al., 2015). Two polymorphisms in NOS3, T→C and G894→T, are the most studied. T→C resides in the promoter region of NOS3 and regulates transcriptional initiation (Nakayama et al., 1999). The CC genotype at T→C is associated with blunted forearm blood flow responses to ACh in hypertensive subjects (Rossi et al., 2003) and no increases in NOS3 mRNA and endothelial nitric oxide synthase (eNOS) protein expression in response to laminar shear stress in endothelial cells from coronary heart patients (Cattaruzza et al., 2004). The G894→T polymorphism in exon 7 of NOS3 results in substitution of glutamate with aspartate at codon 298 (also denoted as Glu298Asp; Marsden et al., 1993). This polymorphism was significantly associated with FMD; TT genotype carriers had higher FMD than GG or GT genotype carriers (Ingelsson et al., 2008). However, these results are not consistent in the literature (Paradossi et al., 2004; Ingelsson et al., 2008). Moreover, in a cohort of 1446 subjects from the Framingham Heart Study, no significant associations were observed between FMD and 18 single nucleotide polymorphisms (SNPs) in NOS3, including T→C and Glu298Asp (Kathiresan et al., 2005). Thus, the effects of NOS3 polymorphisms on endothelial function are variable and might depend on the study population or other genetic or environmental factors.

Polymorphisms in genes regulating NO bioavailability also have been tested for associations with endothelial function. One is the C542→T polymorphism located in exon 4 of the p22phox subunit of NADPH oxidase (CYBA), which decreases O2− production. In >2000 subjects, Fan and colleagues found that allele carriers showed higher brachial FMD (%) than C allele carriers (Fan et al., 2007). Schächinger also reported T allele carriers had greater endothelium-dependent vasodilator responses in epicardial arteries as compared to C allele carriers (Schächinger et al., 2001). However, the polymorphic effect of C542→T on endothelial function is not consistently observed in smaller studies (Schneider et al., 2003; Fricker et al., 2004; Kiliszek et al., 2007; Rafiq et al., 2014). In contrast, polymorphisms in GTP cyclohydrolase 1 (GCH1), the rate-limiting enzyme in the synthesis of eNOS cofactor 6R-tetrahydrobiopterin (BH4), significantly affect endothelium-dependent vasodilation (Antoniades et al., 2008; Liao et al., 2010; Wolkow et al., 2014). Thus, variation in genes affecting NO bioavailability might have clinical implications for treating endothelial dysfunction. However, consistent association of any polymorphism with endothelial function is not yet firmly established.

In addition to genes affecting NO bioavailability and responsiveness, polymorphisms in other vascular biology related genes also are potential modifiers of endothelial vasomotor function. An apolipoprotein A-I mutation (L178P) was associated with impaired FMD and enhanced CVD risk (Hovingh et al., 2004). Similarly, FMD was lower in diabetic patients without angiopathy carrying the e4 allele of apolipoprotein E (APOE, ApoE4) (Guangda and Yuhua, 2003; Guangda et al., 2006). The recent finding that the R952Q variant in apolipoprotein E receptor 2 (ApoER2) and ApoE4 negatively affect NO-mediated endothelial cell repair and eNOS activation substantiated the link between ApoE4 and impaired endothelial function (Ulrich et al., 2014). Furthermore, polymorphisms in paraoxonase 1 (PON1), an antioxidant linked to high-density lipoproteins, have been associated with endothelial dysfunction. Male diabetics with the arginine allele of the Gln192Arg polymorphism in PON1 had reduced FMD compared with male homozygotes for the glutamine allele (Irace et al., 2008). Conversely, variation in PON1 was associated with coronary endothelial dysfunction in women, but not men (Yoshino et al., 2016). Those results suggest that sex, vessel size, and disease status might influence the variant effects of PON1. Further investigation into the role of this gene on endothelial function is warranted. Collectively, these associations suggest genetic regulation of endothelial vasomotor function.
| Study                  | Subject | Age (year) | Measurement | Gene (variant) | Genotype frequency (n) | Polymorphic effect                                      |
|-----------------------|---------|------------|-------------|----------------|------------------------|--------------------------------------------------------|
| Akpinar et al., 2014  | 255 healthy | 35 ± 2    | Baseline FMD | NOS3 (Intron 4 a/b) | aa: 3/ab: 42/bb: 210 | No effect                                              |
| Devri et al., 2005    | 129 hypertension | 20–50     | Baseline FMD | NOS3 (Intron 4 a/b) | aa: 1/ab: 31/bb: 97 | No effect                                              |
| Cottonuzzi et al., 2004| 99 CHD | 62 ± 1    | Responses to ACh in SV | NOS3 (T<sup>−786</sup> → C) | TT: 41/TC: 45/CC: 13 | Vasorelaxation to ACh ↓ in CC genotype               |
| Erbs et al., 2003     | 67 CAD |          | Intra-arterial infusion of ACh and response to ET | NOS3 (T<sup>−786</sup> → C) | TT: 25/TC: 27/CC: 15 | Baseline APV and response to ET ↓ in T allele carriers |
| Fricker et al., 2004  | 72 healthy male | 27 ± 1    | Intravenous infusion of BKN | NOS3 (T<sup>−786</sup> → C) | TT: 27/TC: 32/CC: 13 | No effect                                              |
| Negrao et al., 2010   | 72 healthy male | 18-35     | Baseline FBF and response to ET | NOS3 (T<sup>−786</sup> → C) | TT: 37/TC&CC: 35 | FBF change during exercise ↓ in TT genotype, but no effect on FBF response to ET |
| Paradossi et al., 2004| 118 healthy | 21-45     | Baseline FMD | NOS3 (T<sup>−786</sup> → C) | TT: 43/TC: 58/CC: 17 | No effect                                              |
| Rossi et al., 2003    | 137 hypertension | 49 ± 9    | Baseline FBF | NOS3 (T<sup>−786</sup> → C) | TT: 38/TC: 69/CC: 30 | FBF ↑ in TT genotype                                  |
| Erbs et al., 2003     | 67 CAD |          | Intra-arterial infusion of ACh and response to ET | NOS3 (G<sup>894</sup> → T) | GG: 3/GT: 31/TT: 33 | Baseline APV and response to ET ↓ in G allele carriers |
| Fricker et al., 2004  | 72 healthy male | 27 ± 1    | Intravenous infusion of BKN | NOS3 (G<sup>894</sup> → T) | GG: 29/GT: 28/TT: 15 | No effect                                              |
| Kiliszek et al., 2007 | 44 CAD/QHD | 40-75     | Baseline FMD | NOS3 (G<sup>894</sup> → T) | GG: 17/GT: 25/TT: 2 | No effect                                              |
| Paradossi et al., 2004| 118 healthy | 21-45     | Baseline FMD | NOS3 (G<sup>894</sup> → T) | GG: 43/GT: 57/TT: 18 | FMD ↓ in TT genotype                                  |
| Rossi et al., 2003    | 137 hypertension | 49 ± 9    | Baseline FBF | NOS3 (G<sup>894</sup> → T) | GG: 39/GT: 51/9/TT: 9.1 (%) | No effect |
| Ingelsson et al., 2008| 969 unspecified | Aged 70   | Baseline FMD | NOS3 (23 SNPs) | Only 14th intron A/G is related to EDV (EDV ↑ in heterozygous genotype) |
| Ingelsson et al., 2008| 969 unspecified | Aged 70   | Intra-arterial infusion of ACh | NOS3 (23 SNPs) | Only 14th intron A/G is related to EDV (EDV ↑ in heterozygous genotype) |
| Kathiresan et al., 2005| 1446 unspecified | 62 ± 9    | Baseline FMD | eNOS (18 SNPs) | 4 variants (hCV33219467, rs1800783, rs1800781, rs100731) are related to FMD only in men |
| Akpinar et al., 2014  | 255 healthy | 35 ± 2    | Baseline FMD | ACE (16th intron V/D) | II: 45/ID: 111/DD: 99 | No effect                                              |
| Devri et al., 2005    | 184 healthy | 15-73     | Baseline FMD | ACE (16th intron V/D) | II: 46/ID: 89/DD: 49 | No effect                                              |
| Cottonuzzi et al., 2004| 129 hypertension | 20-50     | Baseline FMD | ACE (16th intron V/D) | II: 23/ID: 63/DD: 43 | No effect                                              |
| Akpinar et al., 2014  | 255 healthy | 35 ± 2    | Baseline FMD | AT1 R (A<sup>1166</sup> → C) | AA: 18/AC: 66/CC: 3 | FMD ↓ in C allele carriers                             |
| Devri et al., 2005    | 129 hypertension | 20-50     | Baseline FMD | AT1 R (A<sup>1166</sup> → C) | AA: 94/AC: 32/CC: 3 | No effect                                              |
| Kiliszek et al., 2007 | 44 CAD | 40-75     | Baseline FMD | AT1 R (A<sup>1166</sup> → C) | AA: 21/AC: 20/CC: 3 | No effect                                              |
| Li et al., 2015       | 483 MI | 65-79     | Baseline FMD | AT1 R (A<sup>1166</sup> → C) | AA: 216/AC: 155/CC: 112 | FMD ↓ in C allele carriers                             |

(Continued)
| Study                  | Subject | Age (year) | Measurement | Gene (variant) | Genotype frequency (n) | Polymorphic effect                                           |
|-----------------------|---------|------------|-------------|----------------|------------------------|-------------------------------------------------------------|
| Fan et al., 2007      | 2058    | 24–39      | Baseline FMD | CYBA (C242→T)   | CC: 1362/CT: 616/TT: 80 | FMD ↓ in CC genotype                                        |
| Fricker et al., 2004  | 72      | 27 ± 1     | Intravenous infusion of BKN | CYBA (C242→T)   | CC: 32/CT: 31/TT: 9    | No effect                                                  |
| Kiliszek et al., 2007 | 44 CAD/CHD | 40–75      | Baseline FMD | CYBA (C242→T)   | CC: 21/CT: 19/TT: 4    | No effect                                                  |
| Ralf et al., 2014     | 72 healthy male | 27 ± 1     | Intravenous infusion of BKN | CYBA (C242→T)   | CC: 32/CT: 31/TT: 9    | FMD ↑ in T allele carriers                                 |
| Schächinger et al., 2001 | 93 CAD and healthy | 40-60s   | Baseline FMD | CYBA (C242→T)   | CC: 32/CT: 31/TT: 9    | No effect                                                  |
| Schneider et al., 2003| 90      | 46 ±14     | Baseline FBF | CYBA (A640→G)   | AA: 18/AG: 17/GG: 7    | No effect                                                  |
| Kiliszek et al., 2007 | 347     | 65 ± 1     | Responses to ACh in SV | GCH1 (3 SNPs)   | OO (non) /XO (het) /XX (homo) | Vasorelaxation to ACh ↓ in X haplotype carriers |
| Liao et al., 2010     | 611     | 40–60s     | Baseline FMD | GCH1 (C903→T)   | CC: 214/CT: 277/TT: 120 | Only rs841 in the 3'-UTR is related to FMD (FMD ↓ in aa genotype) |
| Wolkow et al., 2014   | 182     | 37–72      | Baseline FMD | GCH1 (5 SNP2)   | Log [FMD] ↓ in carriers                                     |
| Hovingh et al., 2004  | 201 healthy | 0.8–90     | Baseline FMD | APO A-I (L178P mutation) | L178P carriers: 54/noncarrier: 147 | FMD ↓ in e4 allele carriers |
| Guang and Yuhua, 2003 | 255 T2DM | 58 ± 8     | Baseline FMD | APOE (e2, e3, e4) | e2/2/e2: 34/e3: 3: 161/e4/3e4: 58 | FMD ↓ in e4 allele carriers |
| Guang et al., 2016    | 144 T2DM | 57 ± 5     | Baseline FMD | APOE (e2, e3, e4) | e2/2/e2: 24/e3: 3: 90/e4/3e4: 30 | FMD ↓ in e4 allele carriers |
| Irace et al., 2008    | 118 Diabetic male | 56 ± 8     | Baseline FMD | PON1 (Q192→R) | QQ: 64/QR:FR: 54 | FMD ↓ in R allele carriers |
| Alves et al., 2013    | 71 healthy male | 19–36   | FBF response to ET | BDKR62 ( exon1 +9→-9) | -9/-9: 17, -9/+9: 34, +9/+9: 20 | FBF response to ET ↑ in -9/-9 genotype |
| Lemos et al., 2016    | 304 healthy male | 19–36   | FBF response to ET | BDNF (Val66Met) | Val66Val: 221/Val66Met: 83 | FBF response to ET ↑ in Val66Met genotype |
| Park et al., 2007     | 47 pre-/stage I hypertension | 50–75 | FBF and response to ET | NFKB1 (t→94→D) | II: 8/ID: 23/OD: 5 | Hyperemic FBF ↓ in DD genotype and its response to ET ↑ in I allele carriers |

CHD, Coronary heart disease; CAD, Coronary artery disease; MI, Myocardial infarction; HC, Hypercholesterolaemia; T2D, Type 2 Diabetes; SV, saphenous veins; ACh, Acetylcholine; BKN, Bradykinin; FMD, Flow-mediated dilation; APV, Average peak velocity; ET, Exercise training; BAD, Brachial artery diameter (mm); FBF, Forearm blood flow; EDV, Endothelium-dependent vasodilation; NOS3, Nitric oxide synthase 3; ACE, Angiotensin converting enzyme; AT1R, Angiotensin II receptor, type 1; CYBA, Cytochrome b-245 alpha chain; GCH1, GTP cyclohydrolase I; APO A-I, Apolipoprotein A-I; APOE, Apolipoprotein E; PON1, Paraoxonase 1; BDKR62, Bradykinin receptor B2; BDNF, Brain-derived neurotrophic factor; NFKB1, Nuclear factor NF-kappa-B p105 subunit.
involves pathways not directly associated with the canonical NO pathway.

**Genome Wide Association Studies**

Over the last decade, genome-wide association studies (GWAS) have been used to identify novel genetic loci underlying CVD and other chronic diseases, but GWAS for endothelial function have been limited. Vasan and colleagues conducted an association study for several cardiovascular traits including FMD (%) and hyperemic flow velocity in 1345 subjects from the Framingham Heart Study using a 100k SNP set (Vasan et al., 2007). They identified several SNPs associated with each trait, including SNPs in cystic fibrosis transmembrane conductance regulator (CFTR) and phosphodiesterase 5A (PDE5A). CFTR encodes a chloride channel expressed in endothelial and vascular smooth muscle cells (Tousson et al., 1998; Robert et al., 2005). PDE5A encodes a cGMP-specific phosphodiesterase that regulates smooth muscle relaxation (Kass et al., 2007). Although those findings have not been replicated, that was the first GWAS to directly investigate endothelial function in a large size sample population, offering a fundamental framework for GWAS of endothelial function.

Subsequently, Yoshino and colleagues performed an association study for coronary vascular responses to ACh, an index of coronary endothelial function, in 643 female and male subjects (Yoshino et al., 2016). They utilized 1536 tag SNPs located in genes previously connected with cardiovascular physiology and pathology. Variants in adenosine A1 receptor (ADORA1) were associated with endothelial dysfunction in the entire cohort, whereas variants in adenosine A3 receptor (ADORA3) and lipoprotein(a) (LPA) had the strongest associations with increased risk of endothelial dysfunction in women only. Their sex-specific results further suggest genetic regulation of endothelial (dys)function might differ between sexes and future studies must consider sex by genotype interactions.

**Genetic Contribution to Endothelial Responses to Exercise Training**

Exercise training is a non-pharmacological means to improve endothelial function. However, there is growing acceptance that phenotypic responses to exercise training are heterogeneous. Green observed a wide range of inter-individual variation in FMD (%) changes after exercise training (Green et al., 2014). Among 182 subjects, 76% exhibited improved FMD, while 24% showed no changes or even decreased FMD after exercise training. Thus, exercise training can exert non-uniform effects on endothelial function among individuals. Hopkins (Hopkins et al., 2012) provided evidence for a genetic contribution to these variable responses. After 8 weeks of aerobic exercise training changes in FMD (%) were highly correlated in monozygotic twins ($r = 0.63$), whereas changes in FMD (%) were not correlated in dizygotic twins ($r = 0.37$). The estimated heritability of training-induced changes in FMD was 0.74, which is significantly higher than estimates of heritability for FMD in non-exercise training studies. Whether genetic influence on endothelial responses to exercise training is also significantly greater remains to be determined.

Because improvements in endothelial function with exercise training can occur primarily through changes in NO signaling, the impact of polymorphisms in NOS3 and related genes on endothelial responses to exercise training has been examined. In coronary artery disease patients, exercise training for 4 weeks improved ACh-induced average peak velocity (APV) in coronary arteries relative to NOS3 polymorphism (Erbs et al., 2003). Patients carrying C allele at T$^{786}$→C had a smaller improvement in APV (~36%) than patients carrying T allele (~81%), whereas a polymorphic effect of G$^{394}$→T was not observed. Similarly, 18 weeks of exercise training increased forearm vascular conductance during handgrip exercise in TT carriers, but not in CT or CC carriers at T$^{786}$→C of NOS3 (Negrao et al., 2010). For other vascular-related genes, there is limited information regarding their role in improved endothelial function with exercise training (Park et al., 2007; Alves et al., 2013; Lemos et al., 2016). The mixed results from those and other association studies imply there is a complicated interaction between genetic factors and exercise on endothelial adaptation to exercise training.

**GENETIC REGULATION OF ENDOTHELIAL FUNCTION IN RODENTS**

**Candidate Gene Studies**

Candidate gene studies in rodents also have focused on genes previously linked to endothelial physiology and pathology. Many of these genes have been reviewed elsewhere (Faraci and Sigmund, 1999), and full discussion is beyond the scope of this review. Typically those studies have been conducted using knockout mouse models to test a direct functional role of a gene in a vascular phenotype or investigate associated-signaling pathways (Babinet, 2000; Hall et al., 2001). They also have been useful in identifying differences in endothelial function throughout the vascular tree (Gongora et al., 2006), but the clinical relevance of complete gene knockout or overexpression is unclear. Recently, mouse models mimicking subtle genetic variation seen in humans have been developed. A single point mutation at the S1176 phosphorylation site on eNOS that increased enzyme activity also improved endothelial function and clinical outcomes in mice (Atochin et al., 2007; Li et al., 2013). Conversely, responses to ACh are attenuated in blood vessels from mice carrying cell specific dominant-negative versions of PPARγ that mimic human mutations in this gene, but only after high fat diet or angiotensin II infusion (Beyer et al., 2008; Hu et al., 2016). Thus, in mice, genetic modifications resembling human polymorphisms or mutations provide proof of concept that small genetic changes can elicit relevant (patho)physiological changes in endothelial vasomotor function without eliciting marked systemic changes or deleterious effects due to complete gene loss or overexpression. These models can facilitate more precise mechanistic investigation of vascular function, but also provide information that is more translatable to human pathophysiology.
TABLE 2 | Strain-dependent differences in endothelial vasomotor function in animals.

| Study            | Species              | Age (wk) | Sex | Vessel type                  | Vasoactive agents          | Responses                                                                 |
|------------------|----------------------|----------|-----|-------------------------------|----------------------------|---------------------------------------------------------------------------|
| Ryan et al., 2002| Mouse A/J, BALb/cJ, C57BL/6J, C3HeBJ, 129P3/J, 129X1/SvJ, and SWR/J | 16 - 22  | Male| Aorta                         | ACh                        | Compared to C57BL/6J, ↓ response in 129P3/J and 129X1/SvJ and ↑ response in A/J |
| Bendall et al., 2002| Mouse MF1, 129sv and C57BL/6J | 8 - 10   | Male| Coronary vessel (Langendorff) and aorta | BK (coronary vessel) and ACh and A23187 (both vessels) | - ↓ response to both BK and ACh in coronary vessels from MF1 - ↓ response to both bradykinin and ACh in aortas from MF1 |
| Chen et al., 2007| Mouse C3HeB/FeJ, RVB/NJ and SJL/J | 16 - 18  | Both| Aorta                         | ACh                        | ↓ response in aortas from SJL/J                                             |
| Kunert et al., 2006| Rat Consomic panel (Substitution of BN onto SS background) | 10       | Male| Aorta                         | ACh                        | Compared to SS parental strain, When fed a low salt (0.4% NaCl) diet, - ↑ sensitivity (EC50) in Chr. 16 and Y consomic strains - ↓ sensitivity in Chr. 9, 13, 20 consomic strains When fed a high salt (4.0% NaCl) diet, - ↑ sensitivity in Chr. 2 and 8 consomic strains - ↓ sensitivity in Chr. 4, 10, 13, 15, 19, 20, and X consomic strains |
| Kunert et al., 2008| Rat Consomic panel (Substitution of BN onto SS background) | 10       | Female| Aorta                         | ACh                        | Compared to SS parental strain, When fed a low salt (0.4% NaCl) diet, - ↓ maximal relaxation to ACh in Chr. 2, 6, 7, 9, 13, 20 consomic strains When fed a high salt (4.0% NaCl) diet, - ↑ maximal relaxation to ACh in Chr. 5, 13 and 17 consomic strains |
| Kunert et al., 2010| Rat Consomic panel (Substitution of BN onto FHH background) | 10       | Male| Aorta                         | ACh                        | Compared to FHH parental strain, When fed a low salt (0.4% NaCl) diet, - ↑ sensitivity in Chr. 3, 4, 5, 11, 12, 14, and Y consomic strains - ↓ sensitivity in Chr. 10 consomic strain - ↑ maximal relaxation to ACh in Chr. 5 consomic strain When fed a high salt (4.0% NaCl) diet, - ↑ sensitivity in Chr. 5 consomic strain - ↓ sensitivity in Chr. 3 consomic strain - ↑ maximal relaxation to ACh in Chr. 6 consomic strain - ↓ maximal relaxation to ACh in Chr. 1 consomic strain |
| Kunert et al., 2010| Rat Consomic panel (Substitution of BN onto FHH background) | 10       | Female| Aorta                         | ACh                        | Compared to FHH parental strain, When fed a low salt (0.4% NaCl) diet, - ↑ maximal relaxation to ACh in Chr. 5, 15 and 17 consomic strains When fed a high salt (4.0% NaCl) diet, - ↑ maximal relaxation to ACh in Chr. X consomic strain |

ACh, Acetylcholine; BK, Bradykinin; BN, Brown Norway rat strain; SS, Dahl salt sensitive rat strain; FHH, Fawn hooded hypertensive rat strain; Chr., Chromosome.
Mouse and Rat Strain Comparisons
An alternative approach for investigating the genetic basis for vascular function is an inter-strain comparison of vascular phenotypes among different inbred rodent strains. Phenotypic diversity across inbred strains permits identification of novel gene(s) responsible for a phenotype via association analysis between phenotype and genotype over the entire genome (Flaherty et al., 2005; Flint and Eskin, 2012). Although no largescale (>10) rodent strain surveys for vascular function have been published, several small studies indicate strain-dependent differences in endothelium-dependent vasorelaxation (Table 2; Bendall et al., 2002; Ryan et al., 2002; Chen et al., 2007). Each study reported impaired responses to ACh in isolated aorta from at least one mouse strain. However, responses to other endothelium-dependent agonists were not consistent, nor were responses in vessels from other vascular beds. Ryan found that two 129-substrains of mice had markedly reduced responses to ACh in isolated aorta, but not carotid arteries, compared with 5 other inbred mouse strains (Ryan et al., 2002). Alternatively, outbred MF1 mice showed impaired responses to ACh in isolated aorta and reduced coronary relaxation responses to bradykinin and ACh in isolated perfused hearts (Bendall et al., 2002). Thus, genetic background influences endothelial function in mice; however, the magnitude might vary depending on the vascular bed. In addition, parallel to lower responses to ACh in aorta from SJL mice, eNOS, and SOD-2 protein expression were lower (Chen et al., 2007). Similarly, impaired responses to ACh in aorta from outbred MF1 mice improved after incubation with the superoxide scavenger Tiron (Bendall et al., 2002). Together these findings imply decreased NO bioavailability may contribute to impaired vasorelaxation responses. However, mechanisms underlying variation in eNOS and antioxidant signaling pathway protein content among inbred mouse strains remain to be elucidated.

Supportive evidence also has been derived from investigations in genetically manipulated rats. A consomic rat panel was created by substituting chromosomes from normotensive Brown Norway (BN) rats onto the background of the Dahl salt sensitive (SS) inbred rat strain (Cowley, 2003). Phenotype comparisons across consomic and inbred parental SS strains afford opportunity to discover chromosomes containing genes contributing to the phenotype of interest. Using that rat consomic panel, aortic rings from strains with substituted chromosomes 16 and Y had greater ACh sensitivity, while aortic rings from strains carrying chromosomes 9, 13, and 20 had reduced sensitivity compared with aorta from inbred parental SS rats (Kunert et al., 2006). These results indicate that chromosomes 9, 13, 16, 20, and Y contain gene(s) responsible for ACh sensitivity. These investigators later utilized a consomic rat panel constructed from BN and Fawn Hooded Hypertensive (FHH) rat strains (Kunert et al., 2010). ACh sensitivity differed for consomic rats of chromosomes 3, 4, 5, 10, 11, 12, 14, and Y compared to parental FHH inbred rats. Only the Y chromosome was identified in both studies as influencing ACh sensitivity, implying chromosomes responsible for endothelial sensitivity to ACh are strain-specific in rats (Kunert et al., 2006, 2010). Collectively, results from animal studies clearly indicate endothelial function has genetic regulation and support comprehensive genomic scans via objective and unbiased hypothesis-free tests to identify novel genomic loci responsible for regulating endothelial function.

FUTURE DIRECTIONS
The endothelium has a critical role in maintaining vascular integrity and protecting against cardiovascular disease. Accumulated data indicate endothelial function is a heritable trait regulated by polygenic factors; however, these genetic factors have not been fully elucidated. Given that single genetic variants generally have only small to modest functional effects, future studies should focus on endothelial function in larger populations (humans or rodents) to facilitate genome wide studies and comprehensively unravel the complex genetic basis of endothelial function. In addition, the majority of studies cited here utilized FMD, a measure of endothelial function in a conduit artery. However, many cardiovascular diseases are associated with endothelial dysfunction in resistance vessels. Collectively, there is less evidence regarding genetic regulation of endothelial function in small vessels and FMD measurements do not always agree with assessment of resistance artery endothelial function via infusion of ACh (Lind et al., 2011). Therefore, studies directly assessing endothelial function in resistance arteries are needed to better understand the genetic regulation of resistance artery endothelial function and its contribution to cardiovascular disease progression.

Because many GWAS identify SNPs outside protein coding regions or in non-coding intervals, the contribution of small non-coding RNA (e.g., lncRNA, microRNA) in modulating endothelial function should be addressed. Emerging evidence suggests microRNA levels are associated with impaired responses to ACh in humans (Widmer et al., 2014) and rodents (Norata et al., 2012; Li et al., 2016). Heritable changes in gene activity and expression also can be the result of epigenetic changes. Recent evidence suggests epigenetic changes such as those induced by histone methyltransferase Set7 are associated with endothelial dysfunction, including impaired FMD in diabetics (Paneni et al., 2015). Furthermore, responses to ACh were impaired in aortic segments from heterozygous lysine-specific demethylase-1 (LSD-1) mice (Pojoga et al., 2011). A polymorphism in LSD-1, which induces histone H3 demethylation, is associated with salt-sensitive hypertension in humans (Williams et al., 2012). Therefore, expanding the search for genetic regulators of endothelial vasomotor tone beyond candidate gene studies could facilitate discovery of modulators of endothelial function and cardiovascular disease.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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