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Genetic modification of hypertension by sGC α_1

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Abstract

Hypertension is an important modifiable risk factor for coronary heart disease, congestive heart failure, stroke, end-stage renal disease, and peripheral vascular disease, but many of the molecular mechanisms and genetic factors underlying the development of the most common forms of human hypertension remain to be defined. Abundant evidence suggests that nitric oxide (NO) and one of its primary targets, the cyclic guanosine monophosphate (cGMP)-generating enzyme soluble guanylate cyclase (sGC), have a critical role in regulating blood pressure. The availability of murine models of hypertension and the revolution in human genetics research (e.g., genome-wide association studies [GWAS]), resulting in the identification of dozens of genetic loci that affect normal variation in blood pressure and susceptibility to hypertension, provide a unique opportunity to dissect the mechanisms by which NO-cGMP signaling regulates blood pressure and to gain important insights into the pathogenesis of hypertension. In this review, we will give an overview of the current knowledge relating to the role of sGC in the regulation of blood pressure, discussing data obtained from genetically modified mouse models as well as from human genetic studies.

The NO-sGC-cGMP signaling system

The gaseous signaling molecule NO, synthesized by a family of three enzymes referred to as NO synthases (NOSs), is a key regulator of numerous (patho)physiological processes, ranging from neurotransmission to gastrointestinal motility, cardiac function, and blood vessel relaxation. NO has many targets, reacting with a variety of intracellular and extracellular molecules, typically via thiol groups or transition metal centers. In the 1980s, and even before endothelium-derived relaxing factor (EDRF) was identified as NO, EDRF released from endothelial cells was shown to activate the hemoprotein sGC in vascular smooth muscle cells (VSMC). It is now well established that sGC is a major target of NO in the cardiovascular system, eliciting many of NO's biological actions. sGC functions as an obligate heterodimer, consisting of an α and a β subunit. NO activates sGC by interacting with the prosthetic heme group associated with the sGC β_1 subunit, thereby disrupting the bond between the heme and a nearby histidine (His105). The subsequent conformational change of the heme-binding domain propagates to the catalytic domain, catalyzing the generation of cGMP from GTP, a fast and tightly controlled process. cGMP can then interact with a variety of effector proteins including cGMP-dependent protein kinases (PKGs) and

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cGMP-regulated phosphodiesterases (PDEs). For a more in-depth discussion of NO-sGC-cGMP signaling mechanisms and biochemistry, we refer the reader to other review papers (Derbyshire and Marletta, 2012; Evgenov et al., 2006; Thoonen et al., 2013).

Although two isoforms of each subunit have been cloned and characterized, only two functional heterodimers of sGC appear to exist in mammals: sGC $\alpha_1\beta_1$ and sGC $\alpha_2\beta_1$ (Mergia et al., 2003). Although these two isoforms are very similar from a biochemical and enzymological point of view, there is evidence suggesting that the subcellular localization of sGC $\alpha_1\beta_1$ and sGC $\alpha_2\beta_1$ is different. While sGC $\alpha_1\beta_1$ appears to be a strictly cytosolic enzyme, the sGC $\alpha_2\beta_1$ is localized at the cell membrane by the interaction of its C-terminal domain with PDZ-containing proteins (Russwurm et al., 2001). Furthermore, sGC $\alpha_1\beta_1$ is by far the predominant isoform in most cell types, including cardiac and aortic tissues, suggesting that the sGC $\alpha_1\beta_1$ heterodimer is the principal cardiovascular sGC isoform (Mergia et al., 2003). However, recent studies suggested that very low levels of cGMP, generated by sGC $\alpha_2\beta_1$, are sufficient to mediate many of NO's cardiovascular effects (Buys et al., 2008; Friebe et al., 2007; Mergia et al., 2006; Nimmegeers et al., 2007; Sips et al., 2011; Vermeersch et al., 2007).

Determination of the chromosomal localization and makeup of the genes encoding the sGC subunits revealed that the sGC α_1 and sGC β_1 genes are co-localized in the same locus in humans (on chromosome 4) and rodents (on chromosome 2 and 3 in rats and mice, respectively), while the sGC α_2 gene is located on a separate chromosome (11 in humans, 8 in rats, and 9 in mice). Extensive characterization of the promoter region revealed the presence of transcription factor binding sites, repressor elements, and enhancer elements that govern expression of (human) sGC α_1 and sGC β_1 (Marro et al., 2008). Some of these elements appear to be mediating previously identified inhibitory effects of NO on sGC expression and therefore have implications for blood pressure regulation. In fact, expressional regulation of sGC has important cardiovascular consequences. For example, sGC expression is reduced in models of pulmonary hypertension, a disease for which NO inhalation is an approved and sGC-dependent therapy (Ichinose et al., 2004).

It is important to note that cGMP is not only produced by sGC but also by a large family of membrane guanylate cyclases (pGCs) that are activated by natriuretic peptides (NPs). cGMP produced by the two GC families (sGC and pGC) potentially has a differential impact on physiology due to differences in spatial and temporal distributions of the cGMP produced. cGMP generated by sGC and pGC is thought to reciprocally regulate blood pressure: impairment of the NO-sGC system results in increased potency of the NP-pGC system and vice versa, thereby maintaining vascular homeostasis (Madhani et al., 2006). Finally, it needs to be highlighted that the cGMP response to NO is not only governed by sGC activity but also by the activity of cGMP-degrading PDEs (such as PDE5, the target for sildenafil) and that cGMP-independent mechanisms have been proposed to mediate other effects of NO as well (Stamler et al., 2001).

Role of the NO-sGC-cGMP signaling system in the etiology of hypertension

Systemic arterial hypertension is one of the most widespread public health problems in the developed world: more than a quarter of the world's adult population is hypertensive and the estimated healthcare cost in the US alone is estimated to be a staggering \$93.5 billion per year (Heidenreich et al., 2011). Hypertension is often associated with abnormal renal salt handling, characterized by a rightward-shift in the relationship between blood pressure and sodium excretion (pressure-natriuresis). It is well established that the renin-angiotensin-aldosterone system (RAAS), which likely evolved to promote salt retention and prevent hypotension in hot climates with limited access to dietary salt, is critical for the short- and long-term regulation of blood pressure and water balance. NP-induced natriuresis is the endogenous salt-excreting system that counter-balances RAAS activity. Defects in both the RAAS and NP-dependent signaling systems can contribute to the development of hypertension, often secondary to kidney dysfunction. Alternatively, primary abnormalities in vascular smooth muscle tone can also underlie increased blood pressure. An example of this alternative mechanism is the observation that mice with a mutated PKGI leucine zipper motif domain developed hypertension associated with systemic vascular dysfunction, without abnormalities in kidney function or salt handling (Michael et al., 2008).

The NO-cGMP-PKG pathway is one of the predominant signaling mechanisms regulating vascular tone. Therefore, it is not surprising that decreased activity of the NO-sGC system has been implicated in cardiovascular pathology, especially in the development of hypertension. The pathway can be perturbed by a variety of mechanisms. The bioavailability of NO can be affected by decreased expression of NOS3 or decreased production of NO by uncoupled NOS3. In addition, uncoupled NOS3 can also be a source of reactive oxygen species, which can scavenge NO. Downstream of NO, decreased expression of sGC may result in impaired NO-cGMP signaling. For example, sGC α_1 and sGC β_1 expression is repressed under pro-inflammatory conditions, an observation that has important implications in cardiovascular diseases characterized by chronic vascular inflammation such as atherosclerosis where sGC may exert anti-atherogenic effects (Marro et al., 2008). Alternatively, sGC can be converted to an NO-insensitive state as a consequence of increased oxidative stress (Stasch et al., 2006) (e.g., resulting from NOS3 uncoupling), a known risk factor for cardiovascular disease.

Ample data suggest that under physiological conditions sGC exists both in reduced (Fe²⁺ or ferrous) and oxidized (Fe³⁺ or ferric) states. The ratio of oxidized/heme-free sGC appears to increase under pathophysiological conditions, resulting in a shift from the reduced NO-sensitive to the oxidized NO-insensitive form. To target the ferric heme or even heme-free state of sGC, a class of direct NO- and heme-independent activators of sGC was developed. BAY 58-2667, also known as cinaciguat, for example, is a sGC activator with unique pharmacological and biochemical properties that is able to induce potent vasorelaxation *ex vivo* and *in vivo*. BAY 58-2667 preferentially activates sGC when the heme is oxidized or missing altogether via a direct interaction with the unoccupied heme-binding pocket or by displacing the weakly bound, oxidized heme. This is in contrast to the action of sGC

stimulator compounds such as BAY 41-2272 and the related BAY 63-2521 (riociguat), which only stimulate activity of reduced heme-containing sGC (Evgenov et al., 2006). In principle, the selectivity of BAY 58-2667 for oxidized/heme-free sGC could be exploited therapeutically, especially in settings where the use of classical NO donors is problematic because of the development of tolerance. The use of a sGC activator could allow for preferential targeting of diseased versus normal blood vessels (Boerrigter et al., 2007), without the possibility of tolerance. Another advantage of targeting sGC is that it bypasses reduced bioavailability of NO, which is commonly associated with endothelial dysfunction. Finally, an additional benefit of targeting sGC directly and selectively with specific activators or stimulators rather than using NO-donor compounds would be that the former likely allows for a more specific approach since the biological actions of NO are not only mediated by cGMP but also by cGMP-independent mechanisms. Further elucidation of the exact role of sGC and its isoforms will impact bench-to-bedside translation of existing sGC-activating compounds and might promote the search for new sGC isoform-specific compounds.

Systemic hypertension in sGC mutant mice

The availability of animal models has permitted researchers to systematically study the etiology of hypertension as it relates to perturbations in the NO-cGMP-PKG pathway (Thoonen et al., 2013). Several knockout models have been developed for sGC, including mice deficient for sGC α_1 , sGC α_2 , or sGC β_1 . In the sGC α_1 -deficient mice (sGC $\alpha_1^{-/-}$ mice), the sGC α_2 -containing isoform remains fully functional and vice versa (Buys et al., 2008; Mergia et al., 2006). In contrast, loss of the common sGC β_1 subunit results in completely sGC-deficient mice (sGC $\beta_1^{-/-}$ mice). Blood pressure is significantly elevated in both male and female sGC $\beta_1^{-/-}$ mice (Friebe et al., 2007). In addition, NO-donor compounds are no longer able to induce relaxation in isolated arteries or decrease blood pressure in sGC $\beta_1^{-/-}$ mice. Similarly, mice with a smooth muscle cell-specific and tamoxifen-inducible deletion of sGC β_1 developed hypertension within 3–4 weeks after induction by tamoxifen, and NO-induced relaxation of aortic smooth muscle was lost after induction by tamoxifen (Groneberg et al., 2010). These results confirm that lack of sGC in smooth muscle cells is sufficient to cause hypertension and that sGC in smooth muscle cells is required for NO-dependent vasorelaxation and to maintain blood pressure homeostasis.

In contrast to the results obtained in sGC $\beta_1^{-/-}$ mice, the ability of NO to induce vasorelaxation, both in larger conduit arteries or smaller resistance vessels, was attenuated but still present in sGC $\alpha_1^{-/-}$ mice (Buys et al., 2012; Mergia et al., 2006; Nimmegeers et al., 2007). Similarly, NO-donor compounds decreased blood pressure in sGC $\alpha_1^{-/-}$ mice and treatment with the NOS-inhibitor N ω -nitro-L-arginine methyl ester hydrochloride (L-NAME) raised blood pressure in sGC $\alpha_1^{-/-}$ mice. On the other hand, the relaxation to the NO-independent sGC stimulators YC-1 and BAY 41-2272 was severely impaired in sGC $\alpha_1^{-/-}$ mice (Nimmegeers et al., 2007), and BAY 41-2272 only lowered blood pressure in wild-type (WT) but not in sGC $\alpha_1^{-/-}$ mice (Buys et al., 2008). The ability of NO to modulate blood pressure and vasorelaxation in sGC $\alpha_1^{-/-}$ mice, together with the inability of NO to affect blood pressure and vasorelaxation in sGC $\beta_1^{-/-}$ mice, indicates that sGC $\alpha_2\beta_1$ can produce sufficient cGMP to mediate the effects of NO in smooth muscle cells. The

observation that NO-independent sGC stimulators and activators preferentially activate sGC $\alpha_1\beta_1$ (Haase et al., 2010) likely explains why BAY 41-2272 did not have an effect on blood pressure in sGC $\alpha_1^{-/-}$ mice. Results from two independent studies have shown that the amount of cGMP produced by sGC $\alpha_2\beta_1$ in response to NO and in the absence of sGC $\alpha_1\beta_1$ is small and virtually undetectable: while stimulation of WT aortic rings with an NO-donor compound resulted in a 100-fold increase in cGMP levels, no significant increase in cGMP levels could be detected in sGC $\alpha_1^{-/-}$ aortic rings, even though they relaxed in response to NO (Mergia et al., 2006; Nimmegeers et al., 2007). These data suggest that very small amounts of cGMP are sufficient to cause vasorelaxation. Where on a subcellular level this small pool of cGMP is generated by sGC $\alpha_2\beta_1$ and whether it signals through the same downstream targets as the substantially larger amounts of cGMP generated by sGC $\alpha_1\beta_1$ in vascular smooth muscle cells remains to be determined. It is also conceivable that sGC α_2 has different effects on cardiovascular function and/or a different subcellular localization in the absence of sGC α_1 . Consistent with the observations that sGC $\alpha_2\beta_1$ is expressed at low levels in the vasculature and is only responsible for a small amount of NO-induced cGMP production, mice lacking sGC α_2 were found to have normal blood pressure levels (Mergia et al., 2006).

Gender modulates blood pressure in sGC $\alpha_1^{-/-}$ mice

The interpretation of the role of sGC $\alpha_1\beta_1$ in the regulation of blood pressure was complicated by the divergent results obtained in male and female sGC $\alpha_1^{-/-}$ mice: male but not female sGC $\alpha_1^{-/-}$ mice bred on a 129S6/SvEvTac background (sGC $\alpha_1^{-/-}$) develop hypertension (Buys et al., 2008). Orchiectomy or treatment with an androgen receptor antagonist prevented hypertension in male mice, and chronic testosterone treatment increased blood pressure in ovariectomized female sGC $\alpha_1^{-/-}$ mice (Buys et al., 2008), suggesting that NO-cGMP signaling has gender-specific and testosterone-dependent effects on blood pressure.

Several other studies had already suggested that NO-cGMP signaling has a greater role in regulating blood pressure in male than in female mice. In one strain of NOS3-deficient mice (NOS3 $^{-/-}$ mice) both male and female mice were hypertensive (Li et al., 2004; Shesely et al., 1996), while in another NOS3 strain (Huang et al., 1995) only male but not female mice develop hypertension (Scotland et al., 2005) at 12 weeks of age (Kubis et al., 2002). Similarly, increased blood pressure was observed in male but not female PKGI-deficient mice (Koeppen et al., 2004; Pfeifer et al., 1998). Taken together, these data suggest a gender-specific impact of NO-cGMP signaling on cardiovascular function.

Interestingly, the gender-specificity of the blood pressure phenotype in sGC $\alpha_1^{-/-S6}$ mice was not accompanied by gender-specific differences in vascular reactivity, assessed *ex vivo* in organ bath studies. The relaxation of aortic and femoral artery rings induced by an NO-donor or acetylcholine appeared to be similarly attenuated in both hypertensive male and normotensive female sGC $\alpha_1^{-/-S6}$ mice (Nimmegeers et al., 2007). These findings suggest that impairment of NO-induced vasodilation does not necessarily result in high blood pressure. This discrepancy may be due to a gender-specific imbalance in the relative contribution of other signaling molecules to *in vivo* vascular reactivity. Possible candidates

include circulating hormones (e.g., testosterone), endothelium-derived relaxing factors (e.g., endothelium-derived hyperpolarization factor, EDHF (Scotland et al., 2005)), and/or contracting factors (e.g., 20-HETE). It is conceivable that one or more of these factors exacerbates or compensates for the vascular dysfunction associated with impaired NO-cGMP signaling in male or female $sGC\alpha_1^{-/-S6}$ mice, respectively.

The clinical importance of gender differences in cardiovascular disease is underscored by epidemiologic studies which have identified male gender as an important risk factor for developing hypertension: men have higher systolic and diastolic blood pressure than women beginning in adolescence and throughout adulthood (Roger et al., 2012). After menopause, blood pressure increases in women to even higher levels than in men (Reckelhoff, 2001), indicating that estrogen likely has anti-hypertensive effects. The gender-related differences in blood pressure observed in sGC mutant mice could provide further clues regarding the gender-specific aspect of the pathogenesis of hypertension.

Genetic modification of blood pressure in $sGC\alpha_1^{-/-}$ mice

Animal models of hypertension are well suited to provide the necessary tools to help define the nature of genetic alterations underlying human essential hypertension. In contrast to human studies for example, there is no need to control or adjust for confounding factors (diet, lifestyle, environment, and genetic complexity) when performing genetic mapping studies in animals, especially inbred rodents. Variation in the genetic makeup of laboratory mouse strains has been shown to affect the phenotypes of mice carrying various knockout alleles (Wade and Daly, 2005) and genetic background has been recognized to influence a wide variety of cardiovascular phenotypes. For example, deficiency of the gene coding for angiotensin-converting enzyme (ACE) 2 was associated with a modest increase in blood pressure in mice on the C57BL/6J (B6) background but had no effect on blood pressure in 129S6 (S6) mice (Gurley et al., 2006). NOS inhibition, on the other hand, had a more profound effect on renal blood flow in S6 mice than in B6 mice (Lum et al., 2004). Different genetic backgrounds might also explain the different cardiovascular phenotype observed in two independently-developed strains of NOS3-deficient mice (Huang et al., 1995; Shesely et al., 1996).

Similarly, a strain-specific difference in blood pressure was detected in $sGC\alpha_1^{-/-}$ mice: male $sGC\alpha_1^{-/-}$ mice bred on a S6 background ($sGC\alpha_1^{-/-S6}$) develop fulminant hypertension (~20–35 mmHg higher than WT mice as measured both invasively in anesthetized mice and using telemetry and tail-cuff plethysmography in awake mice) (Buys et al., 2008). In contrast, no hypertension was observed using invasive hemodynamic measurements in the same strain of $sGC\alpha_1^{-/-}$ mice rederived on a B6 background ($sGC\alpha_1^{-/-B6}$), suggesting the existence of genetic modifiers of the hypertensive effects associated with $sGC\alpha_1$ -deficiency (Buys et al., 2012) (Fig. 1). In another study, in an independently generated strain of $sGC\alpha_1^{-/-B6}$ mice, $sGC\alpha_1$ -deficiency resulted in a moderate (~7 mmHg in awake mice measured by tail-cuff) elevation of blood pressure (Mergia et al., 2006). Relaxation of aortic rings and mesenteric arteries in response to acetylcholine was attenuated in both $sGC\alpha_1^{-/-S6}$ and $sGC\alpha_1^{-/-B6}$ mice as compared to WT mice. However, the response to acetylcholine was attenuated to a greater extent in arteries isolated from $sGC\alpha_1^{-/-S6}$ than in those from

sGC $\alpha_1^{-/-}$ B6 mice (Buys et al., 2012). Together, these data suggest that sGC α_1 -deficiency affects vascular function more in S6 mice than in B6 mice, which raises the possibility that a greater impairment of vascular function in sGC $\alpha_1^{-/-}$ S6 mice contributes to the strain-specific hypertension in sGC α_1 -deficient mice.

The availability of sGC $\alpha_1^{-/-}$ mice on two different inbred backgrounds with very specific cardiovascular phenotypes allowed for the identification of quantitative trait loci (QTLs) affecting blood pressure in the setting of impaired NO-cGMP signaling. In a linkage analysis study of the F2 progeny from a reciprocal sGC $\alpha_1^{-/-}$ B6 $\times$ sGC $\alpha_1^{-/-}$ S6 mice F1 intercross, at least two QTLs were discovered that were associated with blood pressure in the context of sGC α_1 -deficiency: *Hsgcq* on chromosome 1 and a QTL on chromosome 4 (*Chr4D1-QTL*). The effect size of *Hsgcq* was ~10 mmHg (as measured invasively), accounting for ~30–50% of the observed hypertension in sGC $\alpha_1^{-/-}$ S6 mice (~20–30 mmHg) (Buys et al., 2008, 2012), suggesting that other modifier genes (e.g., in *Chr4D1-QTL*) contribute to the hypertension. *Hsgcq* is syntenic with blood pressure-related QTLs in the human and rat genomes and contains the gene coding for renin (Rapp, 2000; Stoll et al., 2000). It was previously discovered that some mouse strains (e.g., B6 and BALB/c) have one renin gene (renin 1^c), whereas others (e.g., S6 and 129P2) have two renin genes, designated renin 1^d and an androgen-responsive renin 2 (Dickinson et al., 1984). Mice carrying two renin genes have a slightly higher blood pressure (Lum et al., 2004). Overexpressing murine renin 2 in rats resulted in hypertension that could be attenuated by a testosterone receptor antagonist (Baltatu et al., 2002), suggesting that androgen receptors are contributing to the hypertension associated with renin 2 overexpression.

Further characterization of the RAAS revealed that the strain-specific hypertension in sGC $\alpha_1^{-/-}$ mice is associated with increased activity of the RAAS in S6 mice (Buys et al., 2012). Plasma angiotensin II (Ang II) levels were similar in WT and sGC $\alpha_1^{-/-}$ mice within the same genetic background but were higher in S6 mice than in B6 mice. A potential blood pressure-modifying role for the RAAS in the development of hypertension associated with impaired NO-cGMP signaling was confirmed by the observation that RAAS inhibition normalized blood pressure and improved endothelium-dependent vasorelaxation in sGC $\alpha_1^{-/-}$ S6 mice. The finding that increased RAAS activity in S6 compared to B6 mice contributed to the development of hypertension in sGC $\alpha_1^{-/-}$ but not WT mice suggests that the blood pressure-modifying role of the RAAS is enhanced in a setting of impaired NO/cGMP signaling. Interestingly, sGC α_1 -deficiency was associated with higher adrenal expression of the aldosterone synthase *CYP11B2* and higher plasma aldosterone levels in S6 mice. This finding suggests that NO/cGMP signaling modulates RAAS activity at the level of *Cyp11b2* gene expression. Increasing evidence suggests that the synthesis or release of vasoactive agents such as Ang II is modulated by NO-cGMP signaling. NO has been shown to downregulate the synthesis of ACE in the endothelium (Takemoto et al., 1997), as well as the Ang II type 1 receptor in vascular smooth muscle cells, thus having the potential to decrease both Ang II production and action (Ichiki et al., 1998). Furthermore, NO-cGMP has been shown to either inhibit (Schnackenberg et al., 1997) or stimulate (Beierwaltes, 2006) renin secretion and activity. In addition, sGC expression and activity was reduced in rats overexpressing murine renin 2 (Jacke et al., 2001). Together, these studies highlight the existence of extensive cross-talk between the NO-cGMP signaling pathway and the RAAS.

In light of the gender-specific hypertension observed in $sGC\alpha_1^{-/-S6}$ mice and the identification of renin as a blood pressure-modifier gene in a setting of impaired NO-cGMP signaling (Fig. 2), it is noteworthy that the RAAS has been implicated in the gender-specific modulation of blood pressure. In a large population study, genetic association of the ACE locus with hypertension was found in men but not in women (O'Donnell et al., 1998). Other studies, in rodents, reported sex differences in the development of hypertension and found that androgens exacerbate hypertension via a mechanism involving the RAAS (Reckelhoff et al., 2000). For example, greater L-NAME-induced hypertension in male than female rats was accompanied by greater plasma renin activity (PRA) in the former and was reversed by orchiectomy (Sainz et al., 2004). The mechanisms by which androgens increase PRA remain to be elucidated but may involve testosterone-mediated upregulation of angiotensinogen (Chen et al., 1992) or ACE expression (Freshour et al., 2002). ACE deficiency decreased blood pressure in male but not female mice (Krege et al., 1995), again suggesting that the RAAS has a more important impact on blood pressure regulation in male mice than in female mice. Together, these data demonstrate that altered activity of the RAAS, possibly by interacting with the NO-cGMP signaling system, may explain at least in part the sexual dimorphisms observed in the cardiovascular system.

Genetic variation in the *GUCY1A3-GUCY1B3* locus influences blood pressure

The multiplicity of genetic factors associated with blood pressure contributes to the complex etiology of essential hypertension. An estimated 31–68% of blood pressure variation can be attributed to the influence of genetic factors (Padmanabhan et al., 2012). Not surprisingly, harnessing the power of genetic studies has dramatically improved our understanding of blood pressure regulation. Interestingly, genes that are involved in the regulation of cGMP are overrepresented in the blood pressure genes identified to date, even more so than renal salt handling genes. Of all the loci that have so far been found to be associated with blood pressure (~60–70 independent loci), only one locus harbors a gene encoding a key protein of the RAAS. By contrast, at least four loci are related to cGMP production. The identification of the genes encoding the ANP and BNP prepropeptides (*NPPA* and *NPPB*) (Newton-Cheh et al., 2009), natriuretic peptide receptor C (*NPR3*) (Kato et al., 2011), and *NOS3* (Johnson et al., 2011) indicates that cGMP is of unique importance to blood pressure regulation (Fig. 2). The clinical relevance of sGC in blood pressure regulation was recently highlighted by the identification of a genetic variant in the *GUCY1A3-GUCY1B3* locus (encoding $sGC\alpha_1$ and $sGC\beta_1$, respectively) that is associated with systolic and diastolic blood pressure (Ehret et al., 2011). In this GWAS, 2.5 million single nucleotide polymorphisms (SNPs) were evaluated for their association with systolic and diastolic blood pressure in ~70,000 individuals of European ancestry. Follow-up of the top signals in a targeted association study in an additional 130,000 individuals yielded the identification of 16 novel loci associated with blood pressure, including the *GUCY1A3-GUCY1B3* locus. SNP rs13139571 (position on chromosome 4: 156,864,963) is a non-coding variant in the last intron of *GUCY1A3*, directly upstream of *GUCY1B3*. The minor A allele of rs13139571 is associated with lower blood pressure. Since GWAS identifies loci defined by correlated SNPs, rather than a single

variant, it remains to be determined what the causal variant is and how it affects sGC expression, activity, and/or responsiveness to NO.

The data described throughout this review illustrate that the combination of high-throughput non-biased genetic approaches and classical candidate-driven approaches represents a unique opportunity to identify genes and pathways that interact to modify blood pressure. Identifying how the interaction of known blood pressure-regulating pathways affects the development of hypertension is particularly critical given the multigenic etiology of essential hypertension. Defining associations between multiple genetic variants, molecular pathways, and blood pressure/hypertension may offer mechanistic insights into the regulation of blood pressure that go beyond an incremental understanding of how one particular molecular pathway regulates blood pressure. Such studies may help geneticists define genetically and etiologically distinct subgroups of patients with essential hypertension based on different risk factors (e.g., gender, age, and ethnicity). Importantly, these studies could potentially lead to the identification of novel (combinations of) targets for interventions to treat hypertension.

Taken together, ample evidence obtained from genetically modified animal models indicates that the NO/sGC/cGMP signaling axis plays a central role in blood pressure regulation. These findings are strongly supported by genetic studies of blood pressure regulation in human subjects, implying that strategies aimed at modulating sGC activity have therapeutic potential as treatment options for hypertensive disorders.

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Fig. 1.

Gender and genetic background modify the blood pressure phenotype of sGCα₁^{-/-} mice. Systolic blood pressure (SBP) was measured invasively in anesthetized male and female wild-type (WT) and sGCα₁^{-/-} mice on a C57BL/6 and 129S6 genetic background. *** $P < 0.001$ versus WT mice of the same gender and genetic background. Data were compiled from previously published studies (Buys et al. 2008, 2009, 2012).

Fig. 2.

Risk factors in the NO-cGMP axis for the development of hypertension. Multiple targets involved in the generation of cGMP have been identified as important modulators of blood pressure. GWAS studies demonstrated that genomic loci coding for NOS3, ANP/BNP, NPR3, and sGC $\alpha_1\beta_1$ (indicated with red starburst) are associated with blood pressure. Animal studies indicated that testosterone and the RAAS modify blood pressure in the setting of impaired cGMP signaling, leading to increased risk for the development of hypertension.