

**Phenotypic Consequences of a Genetic Predisposition
to Enhanced Nitric Oxide Signaling**

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Abstract

Background: Nitric oxide signaling plays a key role in regulation of vascular tone and platelet activation. Here, we seek to understand the impact of a genetic predisposition to enhanced nitric oxide signaling on risk for cardiovascular diseases, thus informing the potential utility of pharmacologic stimulation of the nitric oxide pathway as a therapeutic strategy.

Methods: We analyzed the association of common and rare genetic variants in two genes that mediate nitric oxide signaling [Nitric Oxide Synthase 3 (*NOS3*) and Guanylate Cyclase 1, Soluble, Alpha 3 (*GUCY1A3*)] with a range of human phenotypes. We selected two common variants (rs3918226 in *NOS3* and rs7692387 in *GUCY1A3*) known to associate with increased *NOS3* and *GUCY1A3* expression and reduced mean arterial pressure, combined them into a genetic score, and standardized this exposure to a 5 mm Hg reduction in mean arterial pressure. Using individual-level data from 335,464 participants in the UK Biobank and summary association results from seven large-scale genome wide association studies, we examined the effect of this nitric oxide signaling score on cardiometabolic and other diseases. We also examined whether rare loss-of-function mutations in *NOS3* and *GUCY1A3* were associated with coronary heart disease using gene sequencing data from the Myocardial Infarction Genetics Consortium (n=27,815).

Results: A genetic predisposition to enhanced nitric oxide signaling was associated with reduced risks of coronary heart disease [OR 0.37 95% CI 0.31, 0.45; $p=5.5 \times 10^{-26}$], peripheral arterial disease (OR 0.42 CI 0.26, 0.68; $p=0.0005$) and stroke (OR 0.53 CI 0.37, 0.76; $p=0.0006$). In a mediation analysis, the effect of the genetic score on decreased coronary heart disease risk extended beyond its effect on blood pressure. Conversely, rare variants that inactivate the *NOS3* or *GUCY1A3* genes were associated with a 29 mm Hg higher systolic blood pressure (CI 14, 44 mm Hg; $p=5.6 \times 10^{-5}$) and a three-fold higher risk of coronary heart disease (OR 3.03 CI 1.29, 7.12, $p=0.01$).

Conclusions: A genetic predisposition to enhanced nitric oxide signaling is associated with reduced risks of coronary heart disease, peripheral arterial disease and stroke. Pharmacologic stimulation of nitric oxide signaling may prove useful in the prevention or treatment of cardiovascular disease.

Clinical Perspective

What is new?

- A genetic predisposition to enhanced nitric oxide signaling, as mediated by common variants in the *NOS3* and *GUCY1A3* genes, was associated with lower blood pressure and a reduced risk of coronary heart disease, peripheral arterial disease and stroke.
- Rare variants that inactivate either the *NOS3* or the *GUCY1A3* gene were associated with increased blood pressure and higher risk of coronary heart disease.

What are the clinical implications?

- The results suggest that pharmacologic stimulation of nitric oxide signaling may be an effective therapy for prevention or treatment of atherosclerotic cardiovascular disease.

Introduction

Nitric oxide signaling is a key regulator of vascular tone, blood pressure and platelet aggregation.^{1,2} Endothelial nitric oxide synthase (eNOS), encoded by the gene *NOS3*, generates nitric oxide in the vascular endothelium (Figure 1).³ Nitric oxide acts as a signaling molecule to activate soluble guanylyl cyclase (sGC), a heterodimeric protein with one subunit encoded by the gene *GUCY1A3*.^{3,4} Cyclic guanosine monophosphate (cGMP) produced by sGC then activates downstream signaling molecules, leading to vasodilation, blood pressure lowering, inhibition of platelet aggregation and other cardiometabolic effects.^{5,6} *NOS3* and *GUCY1A3* are thus key mediators of nitric oxide signaling and its downstream effects (Figure 1).

Previous studies have noted increased blood pressure and atherosclerotic burden in eNOS^{-/-} knockout mice,^{7,8} while loss of sGC promotes thrombus formation.⁹ Common noncoding variants in the *NOS3* and *GUCY1A3* loci associate with blood pressure and coronary heart disease (CHD) in genome wide association studies, consistent with a role of nitric oxide signaling in regulation of arterial blood pressure.¹⁰⁻¹² Furthermore, a rare coding variant in *GUCY1A3* was identified as associating with early-onset myocardial infarction in a German family.⁹ These findings in mice and humans suggest that stimulation of nitric oxide signaling may be a useful therapeutic strategy for prevention of cardiovascular diseases.

DNA sequence variants in therapeutic target genes represent naturally-occurring, lifelong variation of the gene (i.e., experiments of nature). Consequently, if a genetic predisposition to enhanced nitric oxide signaling associates with reduced risk of cardiovascular and other diseases, these results would support the therapeutic hypothesis that pharmacologic stimulation of nitric oxide signaling (e.g., through soluble guanylyl cyclase stimulation¹³) will prevent and/or treat cardiovascular disease. Furthermore, if the association of nitric oxide signaling with coronary heart disease (CHD) risk is partially mediated through blood pressure-independent pathways, stimulation of nitric oxide signaling may represent an approach for CHD prevention independent of current blood pressure lowering therapies.

We therefore sought to use common and rare DNA sequence variants in *NOS3* and *GUCY1A3* to: (1) determine the effects of a genetic predisposition to enhanced nitric oxide signaling on a range of cardiometabolic and other diseases; (2) assess whether the effect of nitric oxide signaling on CHD is primarily mediated through blood pressure; and (3) determine if rare inactivating variants in *NOS3* and *GUCY1A3* that are predicted to reduce nitric oxide signaling associate with higher risk for CHD.

Methods

Study design, data sources and study participants

Study design is shown in Supp. Figure 1. We used individual-level data from 335,464 individuals of European ancestry from the UK Biobank, a large population-based cohort (Supp. Methods A). Characteristics of individuals in UK Biobank are provided (Supp. Table 1). We supplemented this individual-level data with seven genome-wide association study (GWAS) consortia examining blood lipids, anthropometric traits, glycaemic traits, diabetes, CHD, migraine and renal dysfunction, all predominantly containing individuals of European descent (Supp. Methods B and Supp. Table 2). Finally, we used gene sequence data from 27,815 participants from the Myocardial Infarction Genetics Consortium and 16,857 participants from the T2D GENES study¹⁴ to examine whether rare variants in the *NOS3-GUCY1A3* pathway associate with blood pressure and CHD (Supp. Methods C).

In our primary analysis, we examined the effect of a genetic predisposition to enhanced nitric oxide signaling on nine different cardiometabolic diseases: CHD, stroke, heart failure, atrial fibrillation, aortic stenosis, peripheral vascular disease, venous thromboembolism, diabetes and chronic kidney disease (Supp. Table 3). We additionally examined the effect of a genetic predisposition to enhanced nitric oxide signaling on 16 quantitative traits (Online Methods A and B): systolic blood pressure, diastolic blood pressure, waist-to-hip ratio adjusted for body mass index, body mass index, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, fasting glucose, fasting insulin, two-hour glucose, hemoglobin A1C, serum creatinine-estimated glomerular filtration rate (GFR), cystatin-C-estimated GFR, forced expiratory volume in 1 second (FEV1) and the ratio of forced expiratory volume in 1 second to forced vital capacity (FEV1/FVC). All traits were standardized (that is, reported in standard deviation (SD) units) to facilitate comparisons among traits (Supp. Methods B). Using the UK Biobank cohort, we conducted a phenome-wide association study for 26 additional diseases, including endocrine, renal, urological, gastrointestinal, neurological, musculoskeletal, respiratory and neoplastic disorders (Supp. Table 3).

Analysis of the UK Biobank data was approved by the Partners Health Care institutional review board (protocol 2013P001840; application 7089). Informed consent was obtained from all participants by the UK Biobank.

*Common variants in *NOS3* and *GUCY1A3**

We leveraged common variants in the *NOS3* and *GUCY1A3* loci to characterize the effects of a genetic predisposition to enhanced nitric oxide signaling. Two common variants were selected as instruments for a genetic predisposition to enhanced nitric oxide signaling: a promoter variant of *NOS3* (rs3918226; minor allele frequency 8%) and an intronic variant of *GUCY1A3* (rs7692387; minor allele frequency 19%; Supp. Table 4). These variants were selected because: (1) they have been robustly associated with blood

pressure (a downstream effect of nitric oxide signaling) and (2) they are located within the endothelial nitrate synthase-soluble guanylyl cyclase nitric oxide signaling pathway.^{15,16} Furthermore, the minor allele of rs7692387 has also recently been characterized to reduce *GUCY1A3* expression via disruption of a ZEB1 transcription factor site.¹⁷ We examined the effect of these variants on *NOS3* and *GUCY1A3* expression levels in aortic and lung tissue in the Genotype-Tissue Expression project database¹⁸ (Supp. Methods D) and the effect of these variants on mean arterial pressure in UK Biobank.

To examine the effects of increased nitric oxide signaling on cardiometabolic and other traits, we pooled rs3918226 and rs7692387 into an additive genetic score (individuals had 0 to 4 risk alleles). We derived this score by multiplying the number of risk alleles by the association of each allele with mean arterial pressure in UK Biobank (0.68 mm Hg for rs3918226 and 0.32 mm Hg for rs7692387). For example, if an individual had two risk alleles for rs3918226 and one risk allele for rs7692387, the genetic score was calculated as $2 \times 0.68 \text{ mm Hg} + 0.32 \text{ mm Hg} = 1.68 \text{ mm Hg}$. Individuals missing one variant [either rs3918226 or rs7692387, $n = 6036$ (1.8%)] were imputed at the mean allele frequency for the variant prior to calculation of the genetic score. We standardized the nitric oxide signaling genetic score to a 5 mm Hg reduction in mean arterial pressure, corresponding to the effect of 1.5 mg of riociguat (a pharmacologic stimulator of soluble guanylate cyclase) on mean arterial pressure in a recent randomized controlled trial.¹³ The standardization was performed by dividing the genetic nitric oxide score by 5 mm Hg (e.g. $1.68 \text{ mm Hg} / 5 \text{ mm Hg} = 0.34$). We also report estimates standardized to a 2.5 mm Hg and 10 mm Hg lower mean arterial pressure to clarify the expected reduction in risk of cardiometabolic outcomes with different levels of nitric oxide signaling.

Rare predicted loss-of-function variants in NOS3 and GUCY1A3

Common non-coding variants may influence the expression of several nearby genes.¹⁹ Therefore, to provide complementary evidence that the *NOS3-GUCY1A3* pathway influences blood pressure and CHD risk, we examined whether rare (minor allele frequency <1%) predicted loss-of-function variants in *NOS3* and *GUCY1A3* are associated with systolic blood pressure, diastolic blood pressure and CHD (Supp. Methods C). Predicted loss-of-function variants were defined as: (1) insertions or deletions of DNA that modify the reading frame of protein translation (frameshift); (2) point mutations at conserved splice site regions which alter the splicing process (splice-site); or (3) point mutations that change an amino acid codon to a stop codon, leading to truncation of a protein (nonsense).

For blood pressure, we tested whether presence of a predicted loss-of-function variant in *NOS3* and *GUCY1A3* was associated systolic blood pressure or diastolic blood pressure in the Type 2 Diabetes Genetics Exome Sequencing study ($n = 16,857$). We examined whether predicted loss-of-function variants were associated with systolic and diastolic blood pressure using the Genetic Association Interactive Tool on the Type 2 Diabetes Knowledge Portal.²⁰ We used linear regression, adjusted for age, sex and five principal components of ancestry.

For CHD, we tested whether presence of a predicted loss-of-function variant was associated with CHD in the Myocardial Infarction Genetics Consortium (n = 27,815) study using logistic regression, adjusted for sex, five principal components of ancestry and a dummy variable for each cohort. To examine whether variants in the *NOS3-GUCY1A3* pathway associate with CHD risk, we pooled the effect of predicted loss-of-function variants in *NOS3* and *GUCY1A3* on CHD using inverse variance weighted fixed effects meta-analysis.

Statistical analysis

For UK Biobank, we estimated the association of the nitric oxide signaling genetic score (standardized to a 5 mm Hg decrease in mean arterial pressure) with each outcome using a logistic regression model adjusting for age, sex, ten principal components of ancestry and a dummy variable for array type. For the summary-level data, this approach is equivalent to an inverse variance weighted fixed effects meta-analysis of the effect of each variant on traits or outcome of interest per 5 mm Hg lower mean arterial pressure. Tests for interaction between UK Biobank and summary-level estimates were calculated as the difference in log-transformed relative risks, as previously described.²¹

For our primary outcomes (nine cardiometabolic diseases), we set a Bonferroni adjusted level of significance of $p=0.05/9=0.0056$. For our secondary analyses of 16 cardiometabolic and pulmonary traits and our phenome wide association study of 26 phenotypes, we set a level of significance of $p=0.05/42=0.001$.

To examine whether an observed reduction in risk of CHD was caused by reduced blood pressure, a mediation analysis was conducted. An estimate of the causal effect of systolic blood pressure on CHD risk was derived from a recent genome wide association study (OR 1.21 CI 1.17, 1.24 per 5 mm Hg higher systolic blood pressure; Supp. Methods E).¹¹ This effect was then multiplied by the decrease in systolic blood pressure due to nitric oxide signaling to estimate the decrease in CHD risk mediated by systolic blood pressure. We then subtracted this estimate from the overall estimate of the nitric oxide genetic score with CHD to derive the remaining proportion of CHD risk unaccounted for by a decrease in systolic blood pressure. For example, if the OR for CHD for the genetic score was 0.5, but the OR for CHD from the systolic blood pressure decrease was 0.75, the OR for CHD independent of systolic blood pressure was calculated as $\exp(\log(0.5) - \log(0.75))=0.67$.

All analyses were performed using R version 3.2.3 software (The R Project for Statistical Computing, Vienna, Austria).

Results

In the Genome Tissue Expression project database¹⁸, the C allele of rs3918226 was associated with increased *NOS3* expression in lung tissue (48% higher expression, $p=0.002$) but was not significantly associated with *NOS3* expression in aortic tissue (23% higher expression, $p=0.21$, Figure 2). The A allele of rs7692387 was associated with increased *GUCY1A3* expression in aortic tissue (20% higher expression, $p=0.012$) but was not significantly associated with *GUCY1A3* expression in lungs (7% higher expression, $p=0.20$). As expected for variants that enhance nitric oxide signaling, both rs3918226 and rs7692387 were associated with lower mean arterial pressure among UK Biobank participants [(0.68 mm Hg lower mean arterial pressure ($p=1.3 \times 10^{-26}$) and 0.32 mm Hg lower mean arterial pressure ($p=8.3 \times 10^{-14}$), respectively) (Figure 2)], replicating previously reported associations of these variants with blood pressure at genome wide significance.^{15,16}

When combined into a nitric oxide signaling genetic score, and standardized to a 5 mm Hg reduction in mean arterial pressure, a genetic predisposition to enhanced nitric oxide signaling was nominally associated with improved renal function, as assessed by both cystatin-C-estimated GFR (7.6 ml/in; CI 2.9, 12 ml/min; $p=0.0015$) and creatinine-estimated GFR (2.8 ml/min; CI 0.57, 5.1 ml/min; $p=0.014$; Figure 3).

Enhanced nitric oxide signaling was significantly associated with higher forced expiratory volume in 1 second (0.09 L; CI 0.05, 0.12 L; $p = 5.6 \times 10^{-7}$). We examined whether the association of a genetic predisposition to enhanced nitric oxide signaling with pulmonary function differed by baseline pulmonary function. No evidence of a trend by baseline pulmonary function for FEV1 was observed ($p=0.34$, respectively, Supp. Figure 2). Enhanced nitric oxide signaling was not significantly associated with any other cardiometabolic traits, including glycaemic traits or blood lipids (Figure 3).

The genetic nitric oxide signaling score was significantly associated with three of the nine primary cardiometabolic outcomes. A genetic predisposition to enhanced nitric oxide signaling was associated with reduced risk of CHD (OR 0.36 CI 0.29, 0.46; $p=7.0 \times 10^{-17}$; Supp. Figure 3) in the CARDIoGRAMPlusC4D Consortium data. We replicated this association in UK Biobank participants (OR 0.39 CI 0.29, 0.52; $p=1.1 \times 10^{-10}$), with an overall pooled 63% reduction in CHD risk (OR 0.37 CI 0.31, 0.45; $p=5.5 \times 10^{-26}$; Figure 4).

Beyond CHD, the nitric oxide signaling genetic score was associated with a reduced risk of peripheral arterial disease (OR 0.42 CI 0.26, 0.68; $p=0.0005$). This association persisted in a sensitivity analysis that excluded individuals with concomitant CHD (OR 0.41 CI 0.23, 0.74, $p=0.003$). The genetic score was also associated at a nominal level of significance ($p < 0.05$) with a reduced risk of stroke (OR 0.53 CI 0.37, 0.76; $p=0.0006$), chronic kidney disease (OR 0.46 CI 0.25, 0.83; $p=0.011$), heart failure (OR 0.59 CI 0.38, 0.92; $p=0.02$) and diabetes (OR 0.81 CI 0.67, 0.99; $p=0.037$). In a phenome wide association study of 26 different diseases, enhanced nitric oxide signaling was not significantly associated with any other disease, including a variety of

gastrointestinal diseases, musculoskeletal diseases and cancers (Figure 4). In sensitivity analyses, when standardized to a 2.5 mm Hg or 10 mm Hg lower mean arterial reduction, the nitric oxide signaling genetic score, was associated with a 39% (OR 0.61 CI 0.56, 0.67) and 86% lower risk of CHD (OR 0.14 CI 0.10, 0.20), respectively (Supp. Figure 4).

When an interaction term between the *NOS3* and *GUCY1A3* genetic loci was included, no evidence of an interaction in the association of nitric oxide signaling with systolic blood pressure (p interaction = 0.76) or diastolic blood pressure (p interaction = 0.49) was observed. Similarly, no evidence of an interaction between the *NOS3* and *GUCY1A3* loci was observed for CHD (p interaction = 0.53).

We performed mediation analysis to determine whether the degree to which the change in blood pressure associated with the nitric oxide signaling score explained the protective effect on CHD. Adjustment for the effect on systolic blood pressure led only to a modest attenuation of the association of the nitric oxide signaling genetic score with CHD (OR 0.37 CI 0.31, 0.45 prior to adjustment, OR 0.46 CI 0.38, 0.55 after adjustment; Supp. Figure 5), suggesting that much of the decrease in CHD risk through increased nitric oxide signaling seems to be through pathways other than blood pressure. Indeed, the effect size of rs3918226 (*NOS3*) and rs7692387 (*GUCY1A3*) on CHD deviated substantially from an estimate based on just the systolic blood pressure effects of these variants (Figure 5).

In contrast to common variants that promote increased nitric oxide signaling, we sought to test the hypothesis that rare inactivating variants in *NOS3* or *GUCY1A3* would be associated with increased blood pressure and risk of CHD. 27 participants with predicted loss-of-function variants in *NOS3* or *GUCY1A3* were identified in the T2D GENES study (Supp Table 5). Presence of a predicted loss-of-function variant in *NOS3* or *GUCY1A3* was associated with increased systolic blood pressure (22.8 mm Hg CI 11.7, 33.9, $p = 5.6 \times 10^{-5}$, respectively; Figure 6) and diastolic pressure (9.7 mm Hg; CI 3.6, 15.9 mm Hg, $p = 0.002$, Supp. Figure 6). 27 individuals with predicted loss-of-function variants were identified in Myocardial Infarction Genetics Consortium studies (Supp. Table 6). Predicted loss-of-function variants in the *NOS3-GUCY1A3* pathway were associated with a three-fold higher risk of CHD (OR 3.03 CI 1.29, 7.12; $p = 0.01$; Figure 6).

Discussion

A genetic predisposition to enhanced nitric oxide signaling was associated with reduced blood pressure, improved renal and pulmonary function, and significantly reduced risks of CHD (OR 0.37 CI 0.31, 0.45), peripheral arterial disease (OR 0.42 CI 0.26, 0.68) and stroke (OR 0.53 CI 0.37, 0.76). Mediation analysis suggested that this protective effect is mediated only in part by blood-pressure related pathways. In contrast, mutations predicted to truncate *NOS3* or *GUCY1A3* associated with higher blood pressure and an approximately three-fold higher risk of CHD.

These results permit several conclusions. First, stimulation of nitric oxide signaling may prevent atherosclerotic cardiovascular disease. The use of an oral soluble guanylyl cyclase stimulator has proven effective in the treatment of pulmonary hypertension¹³, reinforcing the potential to target this pathway using a small molecule approach. The 63% and 58% reductions in CHD and peripheral arterial disease observed in this study are likely to be of greater magnitude than what would be observed in a randomized trial of a nitric oxide signaling stimulator, as genetic estimates represent the effect of increased nitric oxide signaling over a lifetime rather than an intervention later in life and for a more limited duration.²² However, the significant and large risk reductions in cardiovascular disease observed in this study lend support to efforts to target nitric oxide signaling in the prevention or treatment of atherosclerotic cardiovascular disease.^{1,23,24}

Second, stimulation of nitric oxide signaling may represent a pathway for CHD prevention that is independent of current approaches. A genetic predisposition to nitric oxide signaling was not associated with lipid, glycemic, or anthropometric traits. Furthermore, the majority of the reduction in risk of CHD with increased nitric oxide signaling appeared to be independent of the variant's impact on blood pressure. Consistent with these findings, a recent functional characterization of the lead *GUCY1A3* variant rs7692387 observed carriers of the risk allele of this variant to have elevated levels of platelet aggregation and increased vascular smooth muscle cell migration upon stimulation of soluble guanylyl cyclase.¹⁷

Third, these results suggest that stimulation of nitric oxide signaling may improve renal and pulmonary function. A genetic predisposition to enhanced nitric oxide signaling was associated with higher glomerular filtration rate as determined by either cystatin C or creatinine, a finding that awaits further confirmation in larger studies. Increased nitric oxide signaling was also associated with improved pulmonary function. Although medications to stimulate of nitric oxide signaling is effective and approved for treatment of pulmonary hypertension^{1,13}, these results suggest that stimulation of nitric oxide signaling may prove useful for other populations with reduced pulmonary function.

Our study has several limitations. First, we used common variants in the *GUCY1A3* and *NOS3* loci to estimate the phenotypic effects of increased nitric oxide signaling. It is possible that these variants may be in linkage disequilibrium with other variants that have phenotypic effects independent of nitric oxide signaling pathways.

However, the common variants were associated with direct measurement of *GUCY1A3* and *NOS3* expression in vascular and pulmonary tissues, and we observed consistent effects of rare, predicted loss-of-function variants in *GUCY1A3* and *NOS3* on blood pressure and CHD, suggesting that the common variants mediated their effects through the *NOS3-GUCY1A3* nitric oxide signaling pathway. Second, the phenome wide association study to determine the full spectrum of associations in the UK Biobank may have been underpowered to detect associations for some diseases. Third, our rare variant analysis was restricted to variants predicted to lead to loss of *NOS3* or *GUCY1A3* function; the prevalence of missense mutations that impair the *NOS3-GUCY1A3* pathway may be much larger than the prevalence of rare, predicted loss-of-function variants observed in this study. Finally, the majority of participants in this study were of European ancestry and as such, these observations need validation in ancestries outside of Europe as ethnic differences in nitric oxide mediated responses have been previously reported.²⁵

In conclusion, a genetic predisposition to enhanced nitric oxide signaling was associated with reduced risks of CHD and peripheral arterial disease and improved renal and pulmonary function. Stimulation of nitric oxide signaling may prove useful for the prevention and treatment of a range of diseases.

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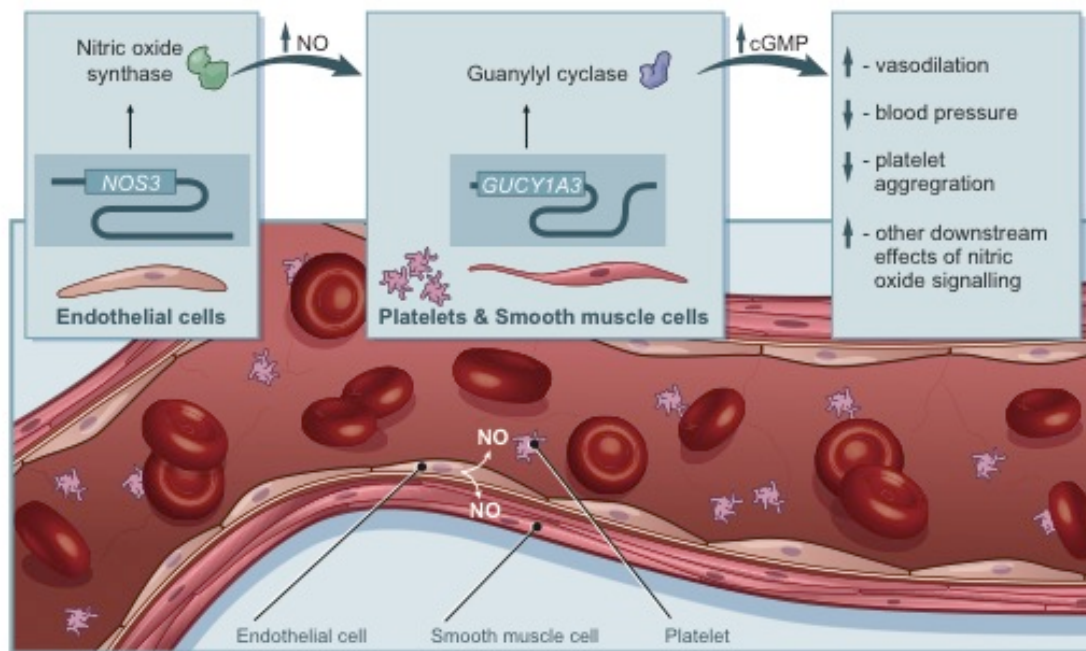


Figure 1. Role of *NOS3* and *GUCY1A3* in nitric oxide signaling. Endothelial nitric oxide synthase, encoded by the gene *NOS3*, generates nitric oxide in the vascular endothelium. Nitric oxide acts as a signaling molecule to activate soluble guanylyl cyclase, a heterodimeric protein with one subunit encoded by the gene *GUCY1A3*. Cyclic guanosine monophosphate produced by soluble guanylyl cyclase then activates downstream signaling molecules, leading to vasodilation, blood pressure lowering, inhibition of platelet aggregation and other cardiometabolic effects.

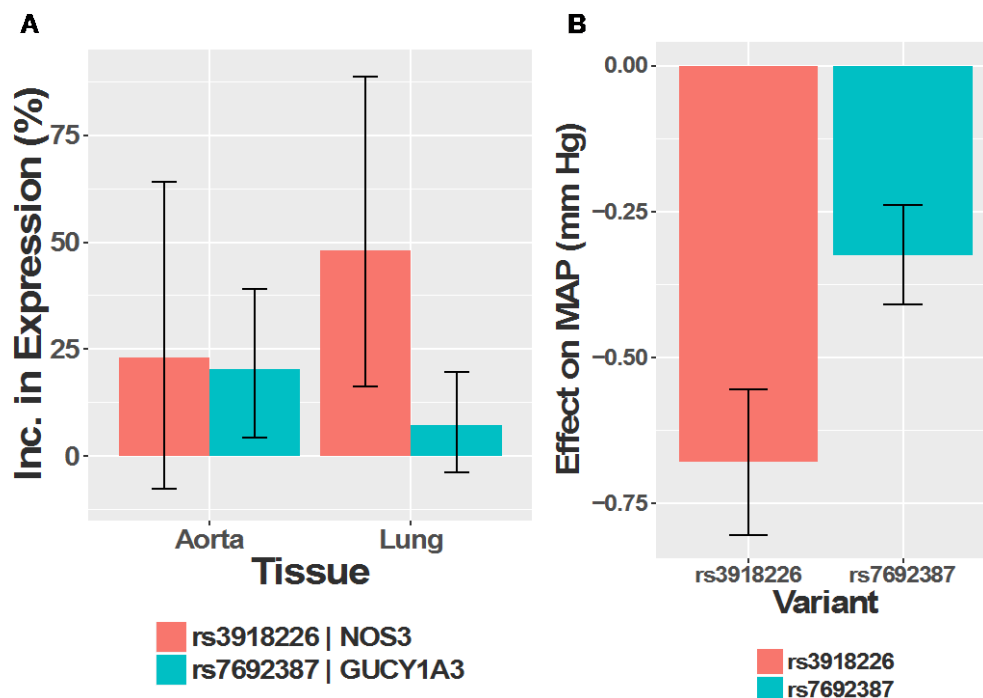


Figure 2. Association of rs3918226 and rs7692387 with (A) *NOS3* or *GUCY1A3* expression levels in aortic and lung tissue and (B) mean arterial pressure among UK Biobank participants. Effect of variants on expression levels were obtained from the Genotype-Tissue Expression project. Effect of variants on mean arterial pressure were derived in UK Biobank using linear regression adjusted for age, sex, ten principal components of ancestry and array type (least-squares means estimates). rs3918226 was associated with significantly elevated *NOS3* expression in lung. rs7692387 was associated with significantly elevated *GUCY1A3* expression in aorta. MAP, mean arterial pressure.

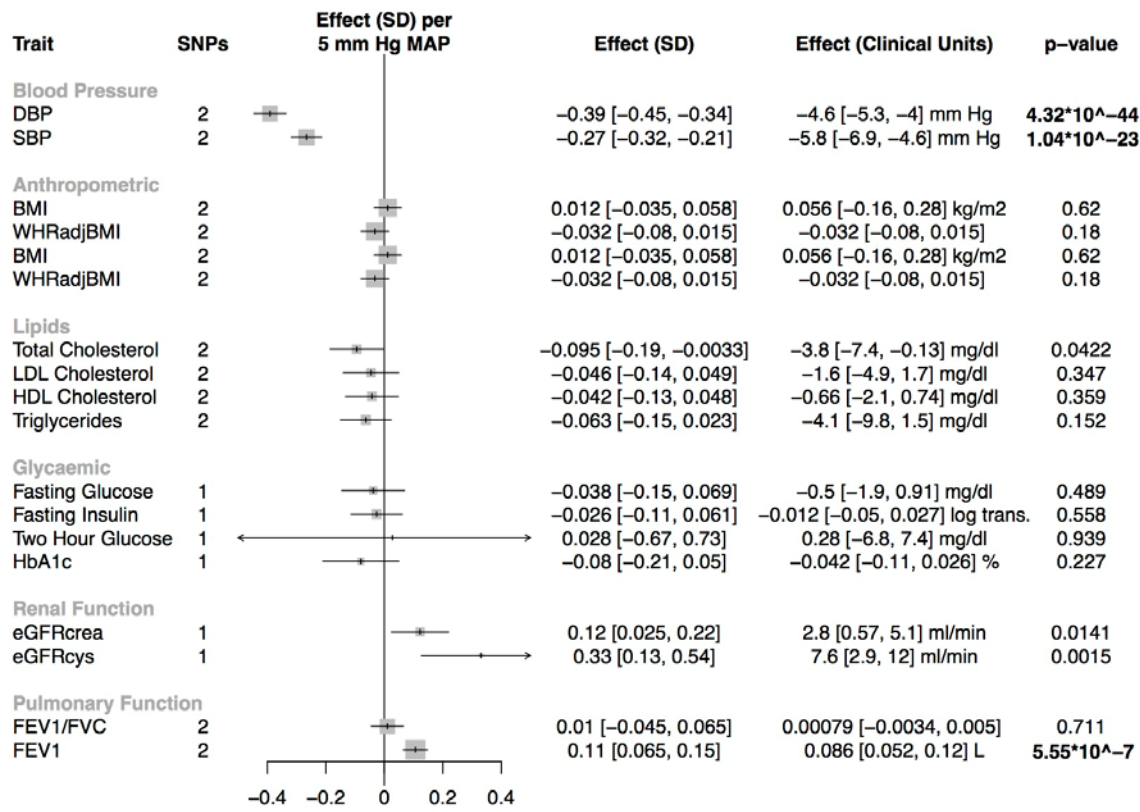


Figure 3. Association of nitric oxide signaling genetic score with cardiometabolic traits (secondary outcomes). Abbreviations: SD, standard deviation; eGFRcrea, serum creatinine-estimated glomerular filtration rate; eGFRcys, cystatin-C-estimated glomerular filtration rate; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WHRadjBMI, waist-to-hip ratio adjusted for body mass index²⁶; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity. P-values that reach Bonferroni-adjusted level of statistical significance ($p < 0.001$) are bolded.

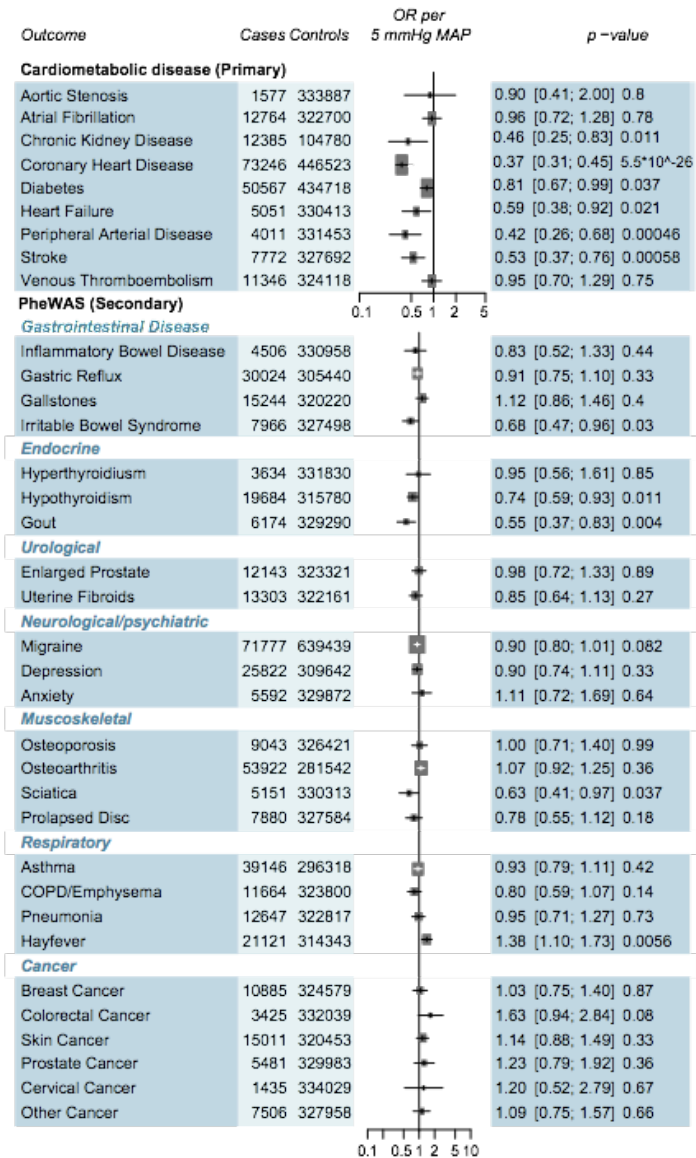
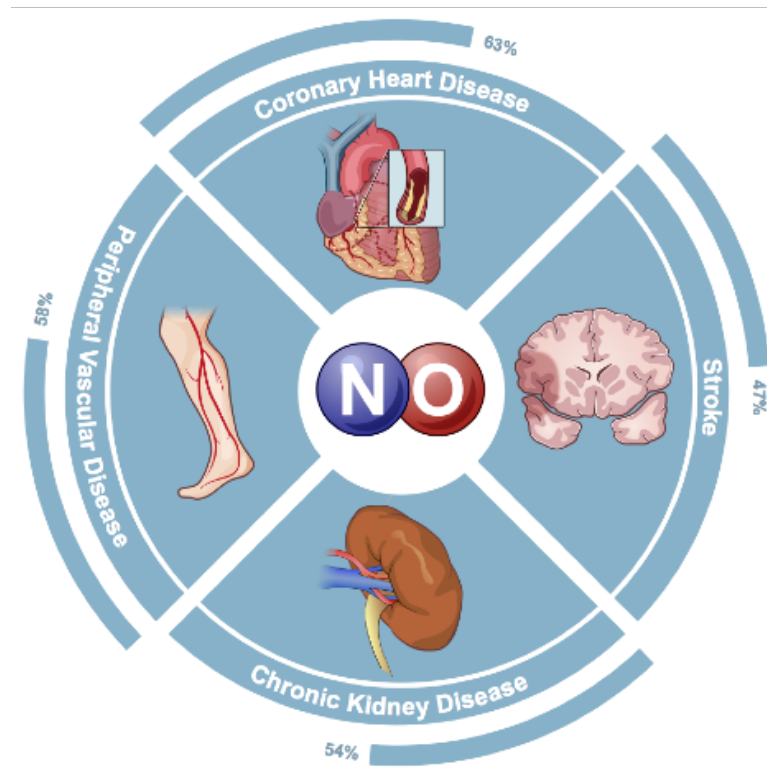


Figure 4. Association of nitric oxide signaling genetic score with cardiometabolic (primary) and other diseases (secondary). Estimates were derived in UK Biobank using logistic regression, adjusted for age, sex, ten principal components and array type, with the exception of chronic kidney disease, which was derived using summary statistics from CKDGen. Estimates for coronary heart disease, diabetes and migraine additionally included summary estimates from CARDIOGRAM, DIAGRAM and IHGC, and were pooled using inverse variance weighted fixed effects meta-analysis. OR, odds ratio; SD, standard deviation; COPD, chronic obstructive pulmonary

disease. CARDIOGRAM, Coronary ARtery DIsease Genome wide Replication and Meta-analysis; DIAGRAM, DIAbetes Genetics Replication And Meta-analysis; PheWAS, Phenome Wide Association Study. Significant p-values are bolded.

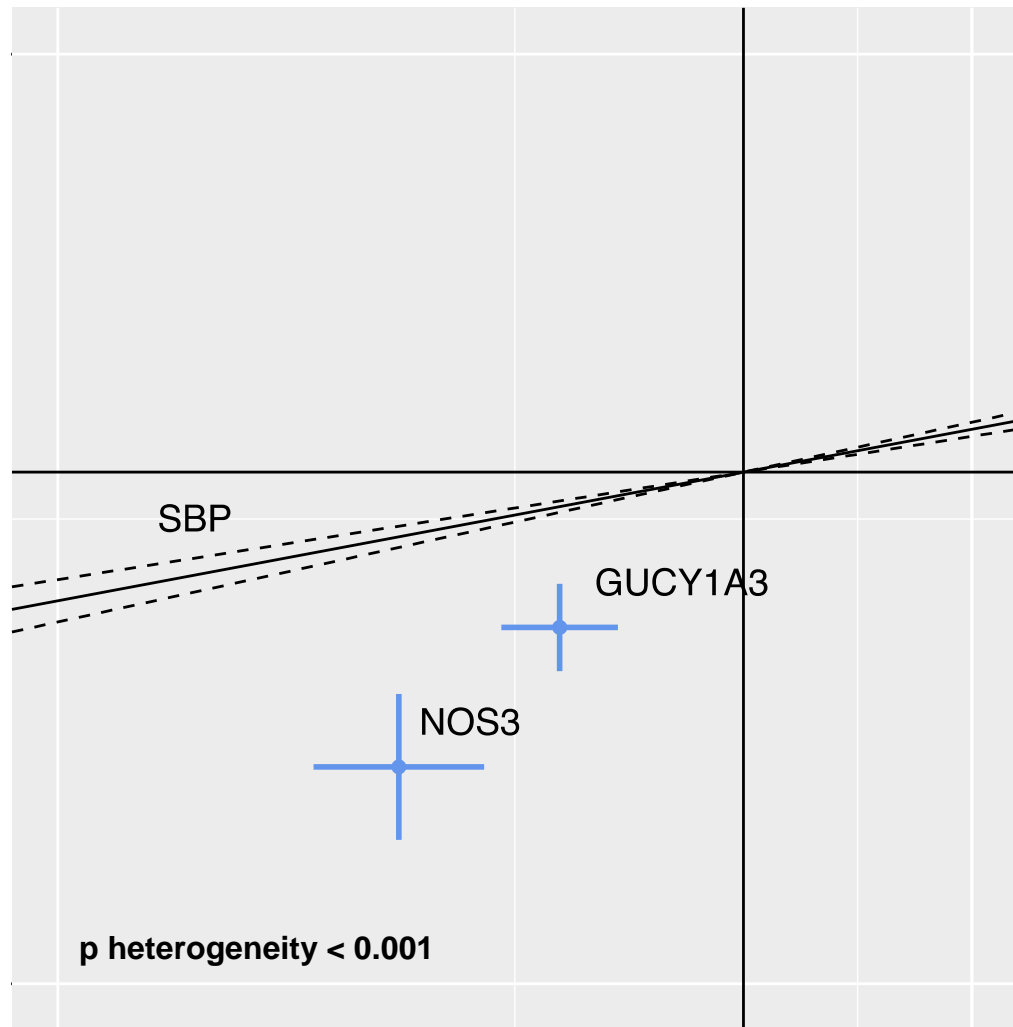
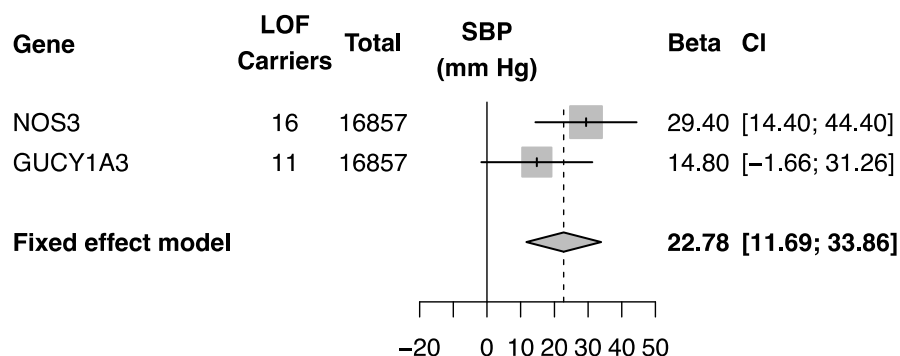


Figure 5. Association of common variants in the *NOS3* (rs3918226) and *GUCY1A3* (rs7692387) loci with systolic blood pressure and coronary heart disease. Solid line represents the estimated effect of systolic blood pressure on coronary heart disease from 54 distinct blood pressure loci from GWAS. A test for heterogeneity comparing the association for the genetic nitric oxide score to other blood pressure loci was significant ($p < 0.001$). Estimates for systolic blood pressure was derived from UK Biobank with adjustment for age, sex and ten principal components. Estimates for coronary heart disease were derived from inverse variance fixed effects meta-analysis of CARDIOGRAM and UK Biobank. Abbreviations: CHD, coronary heart disease; SBP, systolic blood pressure.

A.



B.

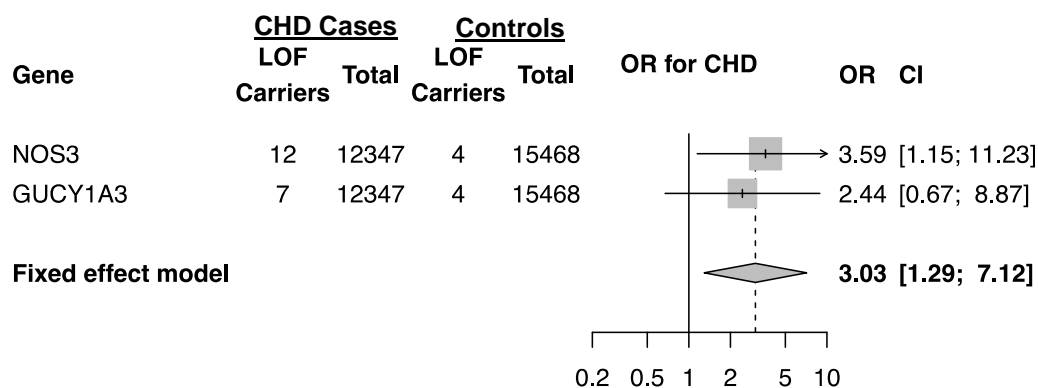


Figure 6. Association of rare, predicted loss-of-function variants in the *NOS3-GUCY1A3* nitric oxide signaling pathway with systolic blood pressure and coronary heart disease. (A) Estimates for systolic blood pressure and diastolic blood pressure from T2D GENES study were derived using linear regression with adjustment for five principal components of ancestry. (B) Estimates for CHD from the Myocardial Infarction Genetics Consortium were derived using logistic regression, with adjustment for sex, cohort and five principal components of ancestry. Abbreviations: LOF, Loss-of-function; OR, odds ratio; CHD, coronary heart disease; SBP, systolic blood pressure