alanine-glyoxylate aminotransferase-2 metabolizes endogenous methylarginines, regulates NO, and controls blood pressure

Ben Caplin, Zhen Wang, Anna Slaviero, James Tomlinson, Laura Dowsett, Mathew Delahey, Alan Salama, The International Consortium for Blood Pressure Genome-Wide Association Studies, David C. Wheeler, James Leiper

Objective—Asymmetric dimethylarginine is an endogenous inhibitor of NO synthesis that may mediate cardiovascular disease. Alanine-glyoxylate aminotransferase-2 (AGXT2) has been proposed to degrade asymmetric dimethylarginine. We investigated the significance of AGXT2 in methylarginine metabolism in vivo and examined the effect of this enzyme on blood pressure.

Methods and Results—In isolated mouse kidney mitochondria, we show asymmetric dimethylarginine deamination under physiological conditions. We demonstrate increased asymmetric dimethylarginine, reduced NO, and hypertension in an AGXT2 knockout mouse. We provide evidence for a role of AGXT2 in methylarginine metabolism in humans by demonstrating an inverse relationship between renal (allograft) gene expression and circulating substrate levels and an association between expression and urinary concentrations of the product. Finally, we examined data from a meta-analysis of blood pressure genome-wide association studies. No genome-wide significance was observed, but taking a hypothesis-driven approach, there was a suggestive association between the T allele at rs37369 (which causes a valine-isoleucine substitution and altered levels of AGXT2 substrate) and a modest increase in diastolic blood pressure (P=0.0052).

Conclusion—Although the effect of variation at rs37369 needs further study, these findings suggest that AGXT2 is an important regulator of methylarginines and represents a novel mechanism through which the kidney regulates blood pressure. (Arterioscler Thromb Vasc Biol. 2012;32:00-00.)

Key Words: alanine-glyoxylate aminotransferase-2 • asymmetric dimethylarginine • hypertension • kidney • NO
Renal Transplant Cohort

Research ethics committee and National Health Service management approval were obtained for the transplant cohort study, which was conducted in accordance with Declaration of Helsinki principles. Written informed consent was obtained from all subjects. The transplant biopsy cohort consisted of patients undergoing protocol biopsy after kidney transplantation as per routine clinical care. Subjects were recruited from the Royal Free Hampstead National Health Service Trust transplant clinic. Patients who refused biopsy or had clinical contraindications to protocol biopsy were not approached. All biopsies were examined by a consultant histopathologist to confirm adequate sampling and the absence of rejection. Contemporaneous blood and urine samples were collected and frozen until further analysis.

Biochemical Measurements

Measurements of routine biochemical variables in patient samples were performed by National Health Service hospital laboratories. Urinary creatinine was measured by Jaffe method. Methylarginine, amino acids, and dimethyl–guanidino valeric acid levels were quantified using liquid chromatography tandem mass spectrometry using a modification of methods previously described. Briefly, an internal standard (deuterium [d7]-labeled ADMA, Cambridge Isotope Laboratories) was added to 50 or 100 µL of biological samples (plasma, urine, tissue lysate, enzyme assay) before precipitation in 5 volumes of methanol. After centrifugation (13,000 rpm for 10 minutes at 4°C), 100 to 200 µL of the supernatant was vacuum dried for 2 hours using a refrigerated vacuum dryer. Liquid chromatography tandem mass spectrometry was performed using an agilent triple quadrupole liquid chromatography tandem mass spectrometry. A hypercarb (Thermo Scientific) chromatography column was used and the mobile phase consisted of 0.1% formic acid, 1% acetonitrile (increasing to 50% between 5 and 10 minutes) over a total run time of 15 minutes. The mass spectrum parameters were as follows: ADMA, m/z: 203.3 to 46.0, collision energy (CE): 12; SDMA, m/z: 203.3 to 70.2, CE: 24; monomethylarginine, m/z: 189.3 to 70.2, CE: 24; arginine, m/z: 175.2 to 50.1, CE: 8; alanine, m/z: 90.1 to 44.1, CE: 4; citrulline, m/z: 176.2 to 86.2, CE: 16; glutamate, m/z: 147.0 to 56.3, CE: 0; dimethyl–guanidino valeric acid, m/z: 202.1 to 70.1, CE: 8; ADGV, m/z: 210.0 to 46.0, CE: 24. Standard curves were generated for each substance of interest. Amino acid standards were obtained as free bases (Sigma) and methylargines as HCl acid salts (Calbiochem). Dimethyl–guanidino valeric acid was generated by hydrolysis of ADMA in methanol at 98°C in the presence of pyruvate with the production confirmed by mass spectra and concentration determined by the stoichiometric disappearance of ADMA, cGMP, aldosterone, and renin were quantified by enzyme-linked immunoassay according to manufacturer’s instructions (Enzo Life Sciences, and R&D Systems for renin). NOx (NO2 and NO3) was measured with a Sievers Nitric Oxide Analyzer as per protocol. Protein concentrations were determined by Bradford assay.

Enzyme Assays

Assays were performed on mitochondrial isolates (Mitochondria Isolation Kit, Thermo Scientific, MA). Mitochondria were suspended in 25-mmol/L HEPES, 0.1-mmol/L EDTA, 12.5-mmol/L MgCl2, 100-mmol/L KCl, 10% glycerol (v/v), 0.1% Nonidet P-40 (v/v), and 5-mmol/L dithiothreitol with protease inhibitors (Roche cOmplete as per instructions). Reactions were performed in a 0.1-mL potassium phosphate reaction buffer with ADMA and an amino acceptor (pyruvate) in 4molar excess. All reactions were performed in the presence of 20-µmol/L pyridoxal-5-phosphate, and the pH was adjusted to 7.5 before the assay. Fifty micrograms of mitochondrial protein was used in a 60-µL reaction with final ADMA concentrations of 10-6, 10-5, 10-4, and 10-3 mol/L. ADGV was quantified and enzymatic production was calculated from the total concentration minus the concentration measured in negative controls (substrate solutions at each ADMA concentration without mitochondrial protein).

NOS activity was determined in whole kidney lysates by quantification of the difference in the disappearance of L-arginine with and without the addition of the NOS inhibitor, NG-nitro-L-arginine.
Kidneys were homogenized in a proprietary reaction buffer (NOS activity kit, Cayman Chemical, Denver, CO) and reactions were performed at 37°C for 1 hour in the presence 1-mmol/L L-arginine. Disappearance of L-arginine was quantified by liquid chromatography tandem mass spectrometry.

**Molecular Biology**

All tissues were immediately immersed in RNA (Qiagen, Hilden, Germany) before being frozen. RNA was extracted using a column system (RNaseasy plus micro, Qiagen) and resulting RNA was reverse transcribed using qScript complementary DNA Synthesis Kit (Quanta Biosciences, MD, human samples) or iScript Supermix (Biorad, Hertfordshire, UK, mouse samples). Primers were designed to amplify complementary DNA from mouse and human DDAH1 and AGXT2, and are listed in the Online Appendix (Table IV in the online-only Data Supplement). Primers for human control genes have been reported previously. For human studies, absolute quantification of complementary DNA was performed on a 7900HT Real-Time PCR System (Applied Biosystems, CA). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) results from the genes of interest were normalized to the 3 most stable of 6 control genes: β-actin (ACTβ), β-2 microglobulin (β2M), and TATA box-binding protein (TBP). For mouse studies, correction was performed using several copies of DNA polymerase II subunit 2 (POL2). All reactions were performed in triplicate.

Tissues for protein analysis were initially snap frozen in liquid nitrogen (and stored at −80°C) before homogenization in PBS with protease inhibitors (Roche complete). Western blots were performed after gel electrophoresis using standard techniques. Goat anti-DDAH1 antibodies were generated as previously described19 and rabbit polyclonal anti-endothelial NOS (eNOS) and phospho-eNOS (Ser1177) antibodies were obtained commercially (Cell Signalling, MA). Rabbit anti-α tubulin, anti-α acetyl-CoA carboxylase antibodies (Cell Signalling), and infrared secondary antibodies (Invitrogen Corporation, NY and Li-Cor, NE) were also obtained commercially. Despite attempts with numerous custom-generated antibodies, no specific detection of AGXT2 in tissue lysates could be achieved.

**Statistical Methods**

All data in text are presented as mean with 95% CIs (or median and interquartile range for non-normally distributed variables). Either
a t test, or Mann-Whitney U test for nonparametric data, was used to examine for differences in continuous variables between groups. Univariate associations between continuous variables were examined using Pearson correlation coefficient (or Spearman rank correlation for skewed data). Log transformation was used where this normalized the distribution of a variable (plasma ADMA and SDMA in human cohort). We used linear regression to adjust for the effects of pre-specified potential confounders on the relationship between plasma methylarginines and AGXT2 expression, that is, age, sex, ethnicity, estimated glomerular filtration rate (eGFR), and DDHA expression. eGFR was calculated using the MDRD equation. Statistical significance was accepted as $P<0.05$. Data were analyzed in Stata version 11 and Prism version 5.

The description of cohorts and the data analysis approach for the meta-analysis of the International Consortium for Blood Pressure Genome-Wide Studies have been reported elsewhere.

**Results**

**AGXT2 Knockout Mice Have Raised ADMA Originating From Reduced Transamination**

Disruption of the AGXT2 gene was achieved through the insertion of a SA-bGeo/Puro cassette in place of exons 8 and 9 (Figure 1A). AGXT2-/- and wild-type groups were generated from heterozygous founders and animals, produced in mendelian ratios, were of normal weight with no observed gross developmental abnormality (Table I in the online-only Data Supplement).

Absence of AGXT2 gene expression and enzyme activity was confirmed in the kidney (Figures 1B and 2). Circulating levels of all 3 endogenous methylarginines were increased and plasma ADGV was substantially reduced in the circulation of AGXT2-/- mice (Figure 1C). No differences were found in the circulating levels of L-alanine, L-arginine, or L-glutamine, although a small but statistically significant increase in L-citrulline was observed (Figure 1D). Whereas significant amounts of ADGV were detected only in wild-type kidney (Figure 1E), changes in ADMA were demonstrated in other organs (Figure 1F).

Next, we examined the consequences of AGXT2 deletion on DDHA1, the enzyme thought to play the major role in ADMA turnover, eNOS in the kidney and aorta. No compensatory increase in the gene or protein (kidney only) expression of DDHA1 could be identified (Figures 3A–3D). Similarly, we could not detect any differences in eNOS expression at the messenger RNA level nor any significant changes in eNOS protein or phosphorylation, although there was a trend toward an increase in phosphorylation in the kidney of AGXT2-/- animals (Figure 3A, 3B, 3E and 3G).

**Kidney Mitochondrial Isolates Demonstrate Enzymatic Transamination of Methylarginines**

To demonstrate enzyme activity at physiological methylarginine concentrations, we performed assays on isolated mitochondria from the kidneys of both wild-type and AGXT2-/- mice. ADGV production was demonstrated after incubation of mitochondrial fractions with ADMA at concentrations between 1 μmol/L and 1 mmol/L without evidence of enzyme saturation (Figure 2). Specific activities at 37°C, pH 7.5, were 3.3 (95% CI 0.8–5.7), 25.7 (95% CI 10.7–40.8), and 259.6 (95% CI 0–540.6) nmoles min⁻¹ ng⁻¹ of mitochondrial protein at substrate concentrations of 1, 10, 100 μmol/L, respectively. Enzymatic activity was supported by the reduction of activity at 4°C and absence of activity in isolates from AGXT2-/- mice. Although previous reports have suggested that the affinity of AGXT2 for ADMA is low ($K_m=14$ mmol/L), these data suggest that the endogenous enzyme deaminates endogenous methylarginines at the prevailing intracellular ADMA concentrations observed in health and disease.

**AGXT2 Knockout Mice Are Hypertensive**

Invasive terminal BPs were measured in male animals aged 8 to 9 weeks, under terminal anesthesia. There was clear evidence of hypertension in the AGXT2-/- mice as assessed by mean BP (Figure 4A), but no differences in heart rate (Figure 4B).

To investigate the mechanism underlying the hypertension in AGXT2-/- mice, we examined the NO pathway, the renin-angiotensin-aldosterone system (because higher renin has been reported in eNOS knockout mice),22 and renal histology. Consistent with the increase in plasma ADMA, there was a decrease in circulating NOX in AGXT2-/- mice (Figure 4C). In knockout animals, there was a suggestion that plasma renin levels were suppressed rather than increased (Figure 4D), although the differences did not reach predetermined levels of statistical significance ($P=0.08$), and aldosterone levels were similar (Figure 4E). Given the increases in endogenous methylarginines observed in other tissues, we also examined downstream NO-dependent signaling but could not identify significant differences in cGMP (Table I in the online-only Data Supplement).

Figure 2. Enzyme activity in isolated mitochondria. Enzyme assays for alanine-glyoxylate aminotransferase-2 (AGXT2) performed in the isolated mitochondrial fraction of kidney homogenates from WT and AGXT2-/- mice at 4°C and 37°C. Mitochondrial isolates were incubated in substrate for 1 h and showed temperature-dependent production of asymmetric dimethyl-guanidinovaleric acid (ADGV) at concentrations of asymmetric dimethylarginine (ADMA) across the physiological range. n=3 for each time point and condition. Error bars: SE of the mean. WT indicates wild-type; AGXT2-/-, AGXT2 knockout.
To further characterize the changes in NO production we examined tissues from the AGXT2-/- mouse ex vivo. NOS activity in kidney homogenates was not reduced under experimental conditions where substrate was present in excess (Figure 4F). There were no differences in isolated aortic rings in responses to PE between genotypes (Figure 4G). As would be predicted, the aortas from AGXT2-/- mice (which do not express AGXT2, Fig 1B) demonstrated no reduction in endothelium-dependent relaxation when free from exposure to high ADMA in the animals’ circulation. Instead, we identified an increase in maximal response to acetylcholine (but no change in EC50) in knockout mice (Figure 4H). We also identified an increase in tension in the aortas from animals of both genotypes in response to ADMA at concentrations similar to the magnitude of the increase in concentration seen in the plasma of AGXT2-/- mice (Figure 4I). There was a similar relaxation in response to sodium nitroprusside in both groups (Figure 4J).

Finally, histological examination of the kidneys revealed no evidence of abnormal development as a consequence of AGXT2 deletion (Figure 4K and 4L).

Renal Allograft AGXT2 Expression Is Inversely and Independently Associated With Plasma ADMA

To investigate if AGXT2 regulates methylarginine levels in humans, we quantified tissue AGXT2 expression and examined the associations with concentrations of substrate and product. The cohort is outlined in Table. None of the allograft biopsies showed active rejection on histological examination. Relative allograft AGXT2 messenger RNA expression levels, determined by qRT-PCR, were strongly inversely associated with both (log) ADMA and (log) SDMA in contemporaneous blood samples (Figure 5A and 5B). AGXT2 expression was also associated with DDAH1 but not DDAH2 levels. Although there was a suggestion of an association between eGFR and DDAH1 expression, there was no association between eGFR and AGXT2 (Table II in the online-only Data Supplement).

To test whether the association between methylarginines and AGXT2 was accounted for by potential confounders, for example, DDAH expression or kidney function, we developed multivariable regression models. After adjustment of the model for potential confounders including DDAH1...
expression, age, sex, ethnicity, and eGFR, the strong inverse association between relative gene expression and circulating methylarginines remained (partial $R^2=0.21; P=0.009$ and partial $R^2=0.20; P=0.011$ for ADMA and SDMA, respectively, in the models including patient factors and DDAH1 expression; see Table III in the online-only Data Supplement). Median urinary ADGV concentrations, the product of AGXT2 metabolism of ADMA, were 0.31 μmol/mmol creatinine (interquartile range 0.16–0.64). There was a correlation between AGXT2 gene expression and urinary concentrations of the ADGV ($n=38, \rho=0.37, P=0.02$). However no association between BP, or BP surrogates (many antihypertensive medications), and AGXT2 expression was observed in this cohort.

Association Between Genetic Variation in the Human AGXT2 Gene and BP
Using data from the International Consortium for BP Genome-Wide Studies, we evaluated sodium nitroprussides spanning up to megabase on either side of AGXT2 on human chromosome 5. There were no results that support an association of this locus and BP using genome-wide thresholds for statistical significance from a meta-analysis of over 69,000 subjects of European ancestry with 2.5 million directly typed or imputed sodium nitroprussides. However, taking a hypothesis-driven approach, we specifically examined the association between genotype rs37369, the V140I substitution associated with an increase in AGXT2 substrate levels, and BP in the same dataset. In an additive model, each minor allele at rs37369 was associated with an increase of $0.31 \pm 0.11$ mm Hg in diastolic BP and an increase of $0.31 \pm 0.18$ mm Hg in systolic BP ($P=0.0052$ and $P=0.076$, respectively).

**Discussion**
In this series of studies, we provide strong evidence that AGXT2 metabolizes endogenous methylarginines in vivo and disruption of this enzyme also leads to reduced NO bioavailability and hypertension. Recent reports have concluded that dysfunction of this enzyme is responsible for hyper $\beta$-aminoisobutyric aciduria in humans by observing an
association between the V140I substitution and urinary levels of this non-methylarginine substrate. Hyper β-aminoisobutyric aciduria has been considered a benign syndrome of altered urinary metabolites, however, to the best of our knowledge no systematic investigation of methylarginines or vascular phenotype has been performed in these patients. Other investigators have shown that this enzyme can metabolize ADMA under experimental conditions, however, our study provides the first convincing evidence that endogenous AGXT2 contributes to the regulation of circulating methylarginines at physiological substrate concentrations.

Allograft protocol biopsies are a relatively unique opportunity to examine the relationships between tissue-level enzyme expression and circulating substrates and enzyme products. The observation that tissue levels of AGXT2 messenger RNA are correlated with urinary concentrations of ADGV (a metabolic product of AGXT2) and ADMA with strongly and inversely associated with levels of the substrates is consistent with a role for this enzyme in the regulation of circulating endogenous methylarginines in humans. Furthermore, the (inverse) association with SDMA (which is not a substrate for the DDAHs), and the finding that the relationship with ADMA is independent of DDAH1 and DDAH2 gene expression, suggest that AGXT2 is a source of significant methylarginine turnover in addition to these better-characterized enzymes. Indeed, we estimated that the variation in AGXT2 expression accounted for approximately 20% of the variation in both (log) ADMA and (log) SDMA after adjusting for other variables (including DDAH1 gene expression) in our patients, a magnitude of change in ADMA that has been associated with cardiovascular events. The lack of associations observed between human AGXT2 gene expression levels and hypertension in this cohort are unsurprising given the small numbers and multiple contributing factors to BP after transplantation.

Consistent with the observational data from patients, disruption of the AGXT2 gene in mice led to an elevation in all 3 endogenous methylarginines. In vitro activity assays confirm that enzymatic degradation of ADMA by AGXT2 occurs in the mitochondrial compartment at physiological pH and substrate concentrations. These data are consistent with evidence from the DDAH1 knockout mouse, where disruption of metabolism also leads to an increase in circulating methylarginines. The relative increase in ADMA is smaller with AGXT2 than with homozygous DDAH1 deletion, perhaps reflecting the limited distribution of this enzyme. Because SDMA is also a substrate for AGXT2, as would be predicted, this was also elevated in the AGXT2-/- mouse. Reductions in the concentration of the product of methylarginine deamination along with absence of enzyme activity in vitro confirm the metabolic consequences of AGXT2 deletion.
The alterations in ADMA identified at sites remote from both AGXT2 expression and dimethyl-guanidovaleric acid detection in this study suggest that there is a significant contribution of this kidney enzyme to plasma methylarginine levels which are then reflected in other tissues. It is interesting that unlike the brain and liver, the increase in ADMA observed in the kidneys of AGXT2-/- mice was not significant. The localization of AGXT2 to the outer medulla (from which venous blood re-enters the circulation without passing through the renal cortex) means that this pathway may predominantly regulate circulating rather than renal interstitial methylarginine levels. We also performed the characterization of related pathways, and although we observed an increase in circulating citrulline in the AGXT2-/- mice, this is unlikely to reflect increased hydrolysis of ADMA because we could identify no compensatory increases in DDAH1 at protein or RNA level. However, the observed increase in aortic maximal response to acetylcholine ex vivo (along with the trend toward increased renal eNOS phosphorylation) strongly suggests a partial compensatory response to chronic inhibition of eNOS in vivo. Furthermore, we could detect no decreases in cGMP in any organ studied. Again, this may reflect compensatory differences in NOS activity, changes in substrate availability (thereby altering arginine to ADMA ratios), or a secondary adaptive response via an increase in the activity of soluble guanylate cyclase. Similar compensatory upregulation of downstream pathways has been reported in previous models of chronic NOS inhibition.

The second important finding of our studies indicates that disruption of AGXT2 leads to hypertension. Knockout of DDAH2 and eNOS also leads to an increase in BP and a reduction in NO bioavailability, through an increase in ADMA in the case of the DDAH1 knockout. In AGXT2-/- mice, the increase in plasma ADMA was smaller than in DDAH1-/- mice (∼25% versus almost a doubling); however, the reduction in plasma NO species seems similar between AGXT2-/-, eNOS-/-, and DDAH1-/- mice (25–50%), and the increase in mean BP (10–20 mm Hg) was also comparable. We investigated the mechanisms underlying the hypertension in AGXT2-/- mice. We could find no evidence of kidney damage or for a renin-angiotensin-aldosterone system-mediated increase in BP. Nor was there the suggestion of differences in NOS activity, changes in substrate availability (thereby altering arginine to ADMA ratios), or a secondary adaptive response via an increase in the activity of soluble guanylate cyclase. Similar compensatory upregulation of downstream pathways has been reported in previous models of chronic NOS inhibition.

We identified the mechanisms underlying the hypertension in AGXT2-/- mice. We could find no evidence of kidney damage or for a renin-angiotensin-aldosterone system-mediated increase in BP. Nor was there the suggestion of a reduction in NOS activity ex vivo, underlined by the observation that ex vivo aortas from AGXT2-/- mice actually show a reduction in NOS activity ex vivo, underlined by the observation that ex vivo aortas from AGXT2-/- mice actually show a reduction in NOS activity ex vivo. We could find no evidence of kidney damage or for a renin-angiotensin-aldosterone system-mediated increase in BP. Nor was there the suggestion of differences in NOS activity, changes in substrate availability (thereby altering arginine to ADMA ratios), or a secondary adaptive response via an increase in the activity of soluble guanylate cyclase. Similar compensatory upregulation of downstream pathways has been reported in previous models of chronic NOS inhibition.

The failure of the gene variation at this locus to reach statistical significance after correction for multiple comparisons in the meta-analysis of genome-wide association studies for BP suggests that polymorphisms in AGXT2 are not responsible for a significant proportion of BP heritability in the European population. The low minor allele frequency (∼8% in whites) of the functional variant in the European population means that the power to detect an association between variation at this locus and hypertension, using genome-wide thresholds for significance, is low. However, taking a hypothesis-driven approach, the V140I substitution, for which there is strong evidence for a functional effect on enzyme activity, was associated with a modest but significant increase in diastolic BP. Further study of the effect of V140I substitution on methylarginine levels in different patient groups and replication of the BP findings in another cohort (of an ethnicity with a higher minor allele frequency) should be undertaken. However, alongside the hypertension demonstrated in the AGXT2-/- mouse, these observations from human cohorts, provide strong evidence for a role for AGXT2 in the regulation of BP. In summary, we confirm the importance of a second route of methylarginine metabolism with functional consequences on cardiovascular homeostasis. Further investigation of this pathway is required to better understand the underlying biology, however, the restricted expression pattern of this enzyme may make it a potentially useful target in the treatment of hypertension.

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Disclosures
None.

References

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Supplement Material

Alanine-Glyoxylate Aminotransferase-2 Metabolises Endogenous Methylarginines, Regulates Nitric Oxide and Controls Blood Pressure

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Supplementary Table I. Additional characteristics of the AGXT2/- mouse

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<th>Weight (grams)</th>
<th>Heart Weight : Tibia Length</th>
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<th>cGMP (pMol/mg protein)</th>
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Summary statistics are mean ± standard deviation. No significant differences between genotypes. n=4-6.
Supplementary Table II. Univariate associations between AGXT2 expression and clinical and molecular biological parameters.

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<td>eGFR (per mL/min/1.73m²)</td>
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<td>relative DDAH1 (per arbitrary unit)</td>
<td>0.5885 (0.3574 to 0.8196)</td>
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Supplementary Table III. Multivariable regression models of the relationship between relative AGXT2 gene expression and methylarginine levels. Covariates/factor were added to the model in the order below (age, sex, black ethnicity, eGFR, DDAH1 expression) and retained where there was an improvement in model fit. Methylarginine levels were log transformed as the distribution was skewed. ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; AGXT2: alanine-glyoxylate aminotransferase-2; DDAH1: dimethylarginine dimethylaminohydrolase; eGFR: estimated GFR. N.B: addition of relative DDAH1 expression to either model does not improve model fit but is shown for information.

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