GSTM1 Deletion Exaggerates Kidney Injury in Experimental Mouse Models and Confers the Protective Effect of Cruciferous Vegetables in Mice and Humans

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ABSTRACT

Background *GSTM1* encodes glutathione S-transferase μ-1 (GSTM1), which belongs to a superfamily of phase 2 antioxidant enzymes. The highly prevalent *GSTM1* deletion variant is associated with kidney disease progression in human cohorts: the African American Study of Kidney Disease and Hypertension and the Atherosclerosis Risk in Communities (ARIC) Study.

Methods We generated a *Gstm1* knockout mouse line to study its role in a CKD model (involving subtotal nephrectomy) and a hypertension model (induced by angiotensin II). We examined the effect of intake of cruciferous vegetables and *GSTM1* genotypes on kidney disease in mice as well as in human ARIC study participants. We also examined the importance of superoxide in the mediating pathways and of hematopoietic *GSTM1* on renal inflammation.

Results *Gstm1* knockout mice displayed increased oxidative stress, kidney injury, and inflammation in both models. The central mechanism for kidney injury is likely mediated by oxidative stress, because treatment with Tempol, an superoxide dismutase mimetic, rescued kidney injury in knockout mice without lowering BP. Bone marrow crosstransplantation revealed that *Gstm1* deletion in the parenchyma, and not in bone marrow–derived cells, drives renal inflammation. Furthermore, supplementation with cruciferous broccoli powder rich in the precursor to antioxidant-activating sulforaphane significantly ameliorated kidney injury in *Gstm1* knockout, but not wild-type mice. Similarly, among humans (ARIC study participants), high consumption of cruciferous vegetables was associated with fewer kidney failure events compared with low consumption, but this association was observed primarily in participants homozygous for the *GSTM1* deletion variant.

Conclusions Our data support a role for the GSTM1 enzyme in the modulation of oxidative stress, inflammation, and protective metabolites in CKD.

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CKD and its cardiovascular disease (CVD) and ESRD consequences are a significant public health burden in the United States.[1] In the African American Study of Kidney Disease and Hypertension (AASK) trial, we found that those with *GSTM1* deletion genotypes *(0/1* or *0/0)* had, respectively, a 1.7- or 2-fold increased risk for the composite outcome of a decline in GFR, commencement of dialysis or all-cause mortality, compared with those with two active alleles [*GSTM1(1/1)*].[2] A similar effect was observed in the Atherosclerosis Risk in Communities (ARIC) study, in which *GSTM1* deletion was associated

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with kidney failure and heart failure in both black and white participants.[3]

A significant portion of humans are homozygous for *GSTM1* deletion [*GSTM1(0/0)*][4] and are therefore completely deficient of the glutathione S-transferase μ-1 (GSTM1) enzyme.[5,6] The proportion of the *GSTM1(0/0)* genotype is population dependent and is approximately 50% in those with European ancestry and 25% in black Americans.[4] The GSTM1 enzyme belongs to a superfamily of glutathione S-transferases (GSTs) and is involved in the elimination of a wide range of electrophiles, both protective and toxic, in the glutathione pathway.[7,8] The noncatalytic domain of GSTM1 has also been shown to suppress the apoptosis signal-regulating kinase (ASK1).[9]

A growing body of evidence suggests that *GSTM1* plays an important role in determining disease susceptibility. The significance of *GSTM1* deletion was first recognized in cancer studies demonstrating that patients carrying the *GSTM1(0/0)* genotype were at increased risk for colon[10] and lung cancers.[11] In subsequent association studies on CVD, participants carrying *GSTM1(0/0)* were shown to have significantly increased risks of hypertension (HTN),[12,13] coronary artery disease/atherosclerosis,[14,15] and stroke.[16,17] Although *GSTM1* deletion is associated with CKD progression, CVD, and cancer (which could be explained by the lack of GSTM1 enzyme for the elimination of toxic electrophiles), *GSTM1(0/0)* genotype has also been shown to enhance the protective effect of intake of cruciferous vegetables, such as broccoli, against lung cancer in multiple populations of European and East Asian ancestries.[18–22]

Over the past decade, the potential benefit of isothiocyanates (ITCs) has garnered significant attention. These compounds are biologically active metabolites of glucosinolates that are abundant in cruciferous vegetables such as broccoli. In particular, the ITC sulforaphane, although not antioxidant itself, has been shown to be highly potent in inducing the phase 2 detoxification enzymes GSTs (including GSTM1), UD-glucuronosyltransferases, sulfotransferases, N-acetyltransferases, and S- and O-methyltransferases *via* activation of the Nrf2 pathway.[23,24] Sulforaphane-rich broccoli contains high levels of glucoraphanin, which is metabolized into sulforaphane. The stronger protective effect of cruciferous vegetable intake in those with *GSTM1(0/0)* genotype may be due to greater total bioavailability of antioxidant-promoting compounds like sulforaphane in these individuals.[25] Very few studies have assessed the effect of sulforaphane in kidney disease. Of these, two studies showed that sulforaphane induced expression of superoxide dismutase and catalase in the liver of spontaneously hypertensive rats,[26] lowered their BP, and decreased renal tissue inflammation.[27]

Multiple studies have demonstrated an association between the loss of *Gstm1* and deleterious effects in animal models. The murine GSTM1 shares approximately 80% amino acid identity with the human GSTM1 and >90% with the rat GSTM1 (basic alignment tool [NCBI BLAST], https://blast.ncbi.nlm.nih.gov/Blast.cgi). In mice, *Gstm1* is a potential candidate modifier of vascular injury. A mouse strain that is more susceptible to renal vascular remodeling with pathologic features resembling human arteriolar nephrosclerosis expressed lower levels of *Gstm1* than the resistant strain, and had faster proliferation and migration of vascular smooth muscle cells (VSMCs).[28,29] In the rat, *Gstm1* is a positional candidate gene in a quantitative trait locus for HTN.[30] *Gstm1* mRNA and protein levels in the kidney were found to be reduced in the stroke-prone spontaneously hypertensive rat (SHRSP), compared with the normotensive congenic and Wistar Kyoto rats,[31] and were inversely correlated with kidney tissue levels of reactive oxygen species.

To further elucidate the pathways by which *GSTM1* deletion affects kidney disease pathophysiology and to identify potential therapeutic targets, we generated a *Gstm1* knockout (KO) mouse line to mimic the common 20 kb deletion variant in humans.[6] Using these mice and wild-type (WT) controls, we studied the role of *Gstm1* in a CKD model and a HTN model. We examined the importance of superoxide in the mediating pathways and of hematopoietic *GSTM1* expression on renal inflammation. Further, we fed mice sulforaphane-rich broccoli powder (SRBP) to determine whether cruciferous vegetables provide a *Gstm1*-dependent protective effect in kidney disease, similar to that observed in lung cancer. The clinical relevance of this effect was also examined in humans, using data from the ARIC study, a community-based prospective cohort.

Significance Statement

GSTM1 encodes a member of a superfamily of antioxidant enzymes, and a highly prevalent *GSTM1* deletion variant is associated with kidney disease progression in two human study cohorts. In this study, the authors demonstrate that *Gstm1* knockout mice exhibit increased oxidative stress, kidney injury, and inflammation in models of CKD and hypertension, and that *Gstm1* loss in the parenchyma but not in bone marrow–derived cells drives renal inflammation. Importantly, consumption of broccoli powder or cruciferous vegetables was protective against kidney disease only in *Gstm1* knockout mice, and was observed mainly in the human participants in the Atherosclerosis Risk in Communities Study who were homozygous for *GSTM1* deletion. These findings suggest that targeting antioxidant therapy specifically in individuals carrying the *GSTM1* deletion variant may be effective in delaying kidney disease progression.

METHODS

Additional details of the methods can be found in Supplemental Appendix 1.

Animal Models

Experiments were carried out in accordance with local and National Institutes of Health guidelines, and the animal protocol was approved by the University of Virginia and University of Rochester Institutional Animal Care and Use Committee. All mice were maintained on a 12-hour light-dark cycle with free access to standard chow and water. The

mouse line carrying the *Gstm1* deletion was generated by the electroporation of embryonic stem cells derived from the 129/SvEv (129S6; Taconic) strain with a targeting construct to disrupt a 20 kb segment of the *Gstm1* locus (Supplemental Figure 1A). Ablation of *Gstm1* was confirmed by Southern blotting and quantitative RT-PCR (Supplemental Figure 1, B and C).

Subtotal Nephrectomy–Induced CKD Model

A subtotal nephrectomy (Nx) was used to induce CKD in the model, as previously described.[32] Briefly, mice were anesthetized with 2%–4% isoflurane. The right kidney was removed and the cranial branch of the left renal artery was ligated, resulting in the infarction of the cranial pole of the left kidney. Mice were allowed to recover for at least 4 weeks before being subjected to any other procedures.

Angiotensin II–Induced HTN Model

The HTN model was induced by the delivery of angiotensin II (AngII; Sigma-Aldrich, St. Louis, MO) at 1000 ng/kg per minutes for 4 weeks *via* Alzet miniosmotic pumps (model 2004; Durect Corporation, Cupertino, CA).

Antioxidant Treatment

Tempol (4-hydroxy-Tempo; Sigma-Aldrich) was administered to mice in drinking water as a 3 mmol/L solution, simultaneous with AngII infusion. Mice treated with SRBP (Natural Sprout Company) had it mixed with powdered chow at a 1:1 ratio. SRBP contains little sulforaphane, but rather glucoraphanin, which is metabolized into sulforaphane in the gut.

BP Monitoring *in vivo*

BP was measured in conscious mice under unrestrained conditions by radiotelemetry (TA11PA-C10; Data Sciences International, St. Paul, MN), as previously described.[33] Briefly, mice were anesthetized with 2%–4% isoflurane and had the radiotelemetry catheter implanted into the left carotid artery. A subcutaneous pouch was made along the right flank of the animal, and the transmitter was placed as caudally as possible. Mice were housed in individual cages on receivers and allowed to recover for at least 7 days after implantation of the radiotelemetry device before measurements were recorded and analyzed using Dataquest A.R.T. 20 software (Data Sciences International). Implantation was performed at least 4 weeks after Nx-CKD or before AngII-HTN initiation. Reported values are expressed as mean±SD.

Urine Measurements

Urine samples were collected over 24 hours from mice placed in individual metabolic cages for assessment of 8-isoprostane and urinary albumin and creatinine, as previously described.[32] Urinary isoprostane was measured using the ELISA kit from Oxford Biomedical Research (Oxford, MI). Urinary albumin and creatinine were measured using the Albuwell M Murine ELISA kit, and Creatinine Companion kit (Exocell, Philadelphia PA).

Measurement of Tissue Superoxide

The lucigenin (9,9′-bis-N-methylacridinium nitrate, M8010; Sigma) assay was modified from previous studies as described,[33] and performed on 3–5 mg of kidney cortical tissue. Luminescence counts were taken five times for 1 minute each, averaged, corrected for background, and normalized to dry tissue weight.

Histopathologic Analyses

Kidney injury was scored blinded using formalin-fixed sections stained with periodic acid-–Schiff as previously described.[32] Digital quantitation of glomerular and mesangial surface areas was performed by light microscopy (Zeiss Axio Imager Z2) using Stereoinvestigator software (BMF Bioscience) as previously described.[34]

Podocyte Isolation and Scratch Assay

Podocytes were isolated as previously described.[35] At confluence, mechanical scraping was performed with a 200 μl pipette tip, creating a rectangular-shaped wound in the podocyte layer. Images of podocytes with the scratched area were taken immediately after wound creation (0 hour) and after 14 hours, using an EVOS XL Core Cell Imaging System at $\times$10 magnification. The images were analyzed using ImageJ software (1.48v; National Institutes of Health) to obtain pixel counts in the scratched area void of podocytes.

Gene Expression Analysis

RNA from frozen kidney tissue was isolated by RNeasy Mini kit (Qiagen) and transcribed to complementary DNA by iScript cDNA synthesis kit (Bio-Rad). Quantitative RT-PCR analysis was performed on an iQ5 system (Bio-Rad) with normalization to *Hprt* as the reference gene. The sequences of all primers used in this study are listed in Supplemental Table 12.

Flow Cytometry

Single-cell suspensions were made from kidney tissue, stained with antibodies for 1 hour at 4°C, and analyzed on a FACSCalibur system (BD Biosciences, San Jose, CA) with an eight-color detector (Cytek Development, Fremont, CA). Live, singlet $CD45^+$ cells (leukocytes) were identified and quantified on the basis of positive staining of respective markers.

BM Transplantation

BM cells were obtained from the femurs and tibias of donor mice. Recipient mice were irradiated and then 0.2 ml of BM cells (5 million cells) were injected *via* the tail vein. Recipient mice were maintained on antibiotics (enrofloxacin) with autoclaved water and food for 5 weeks. Eight weeks post-transplantation, HTN was induced as described above.

Statistical Analyses

Unpaired *t* test, one-way ANOVA with *post hoc* Bonferroni correction, and two-way ANOVA were used as indicated. Differences in Kaplan–Meier survival curves were tested using the

log-rank test. Differences were considered statistically significant when $P<0.05$. All values are presented in the text and figures as mean±SD.

ARIC Study

The ARIC study was a prospective cohort study of 15,792 participants in the United States, aged 45–65 years at visit 1 (1987–1989).The overall analyzed sample in this study included 10,155 participants. The included participants provided informed consent for genetic studies. This study has been approved by the Johns Hopkins Institutional Review Board.

GSTM1 genotypes were determined using exome sequencing reads. The methods have been reported previously.[3] The concordance between this algorithm of calling *GSTM1* deletion using exome sequencing reads and quantitative PCR was 93% (Supplemental Figure 6). We adopted the recessive genetic model, *i.e.*, homozygous deletion [*GSTM1*(0/0)] versus heterozygous deletion [*GSTM1*(0/1)] and no deletion [*GSTM1*(1/1)] combined, as our primary model on the basis of previous findings from lung cancer in humans.[18–20,22] We also performed secondary analysis using the genotypic model, *i.e.*, using the three genotypes as a categorical variable.

Cruciferous vegetable intake of the participants was assessed using a food frequency questionnaire on their dietary intake in the previous year (the Willett 131-item food frequency questionnaire).[36] Among the questions in this assessment, one question was specific to broccoli intake, and another was on cabbage, cauliflower, and Brussels sprouts intake. These vegetables have commonly been considered as cruciferous vegetables containing ITC, a protective electrophile.[37] Cruciferous vegetable intake was categorized into three levels: low (three or fewer times per month), medium (more than three times per month but less than once per week), and high (once or more per week).

Kidney failure was defined as ESRD ascertained using linkage to the US Renal Data System or kidney failure on the basis of International Classification of Diseases, Ninth or Tenth Revision, Clinical Modification (ICD-9-CM/ICD-10-CM) codes for hospitalization or death.[38] Participants were followed from the baseline (visit 1) to December 2013.

Statistical Analyses in the ARIC Study

We compared the baseline characteristics of the participants by three levels of cruciferous vegetable intake, using *t* tests for nonskewed continuous variables, Wilcoxon tests for skewed continuous variables, and chi-squared tests for categorical variables. Our primary analysis evaluated the association between the three levels of cruciferous vegetable intake and kidney failure stratified by *GSTM1* homozygous deletion to determine whether the protective effect of cruciferous vegetable intake was indeed stronger among participants with *GSTM1*(0/0). Kaplan–Meier curves were plotted by the three levels of cruciferous vegetable intake within those with and without *GSTM1* homozygous deletion. Risk of kidney failure was assessed by Cox regression. Model 1 controlled for age, sex, study center, and genetic principal components. Model 2 additionally controlled for clinical risk factors of kidney failure (baseline eGFR, prevalent diabetes, HTN, coronary heart disease, smoking status, body mass index, physical activity, education levels, and total calorie intake). We evaluated these two models combining black and white participants controlling for race within each *GSTM1* homozygous deletion stratum. To determine whether the association between cruciferous vegetable intake and kidney failure was stronger in those with *GSTM1* homozygous deletion, we tested for the interaction between *GSTM1* homozygous deletion and cruciferous vegetable intake. To determine whether the association between cruciferous vegetable intake and kidney failure within the *GSTM1* homozygous deletion strata differed by the two race groups, we tested for race interaction with cruciferous vegetable intake within each *GSTM1* homozygous deletion stratum.

Furthermore, we conducted three sets of secondary analyses. The first set evaluated the association between *GSTM1* genotypes (*0/0, 0/1,* and *1/1*) and kidney failure within the three levels of cruciferous vegetable intake. The second evaluated the main effect of cruciferous vegetable intake on kidney failure. The third evaluated the main effect of *GSTM1* deletion genotype on kidney failure. The covariates of these secondary analyses were the same as the primary analysis.

All Cox regression analyses were conducted using Stata version 14. All other analyses were conducted using R.

RESULTS

Gstm1 Null Mice Have Increased BP and Oxidative Stress at Baseline

Gstm1 KO mice displayed a modest but statistically significantly higher average baseline systolic BP (SBP) of 7 mm Hg compared with WT controls (Figure 1A). We previously reported through knockdown studies that GSTM1 influences oxidative stress in VSMCs *in vitro*.[2,29] Consistent with these earlier findings, $Gstm1^{-/-}$ (KO) mice displayed higher baseline urinary 8-isoprostane levels (marker of oxidative stress) (Figure 1B), suggesting that the deletion directly results in increased oxidative stress *in vivo*. No difference in urinary albumin-to-creatinine ratio (ACR) was observed (Figure 1C), suggesting loss of GSTM1 does not influence the glomerular filtration barrier or tubular resorptive capacity in nondisease state. At 1 year of age, there were no observed differences in mortality or phenotypes between WT and *Gstm1* KO mice (Supplemental Figure 1, D–F).

Deletion of *Gstm1* Results in Poor Survival and Exaggerated HTN and Kidney Injury in the Nx-CKD Model

We previously reported that both Nrf2 and GSTM1 proteins are upregulated in the remnant kidney in WT mice after Nx,[2]

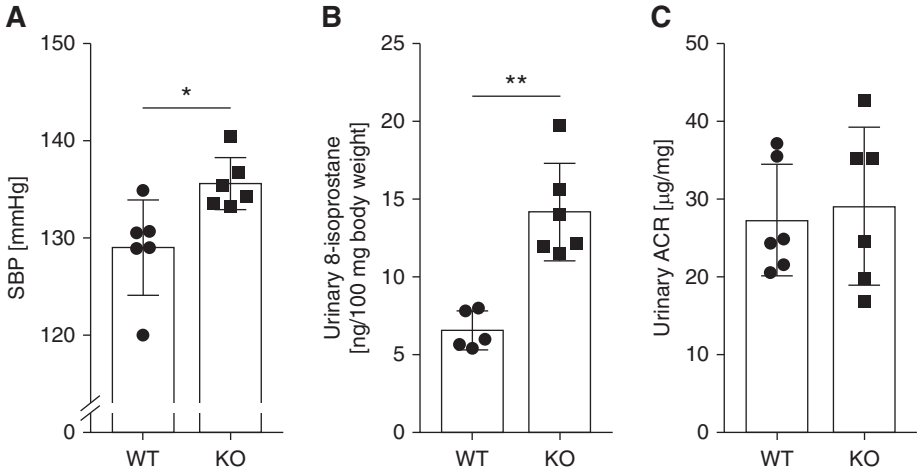

Figure 1. *Gstm1* KO mice have higher BP and renal oxidative stress at baseline. (A) SBP measured by radiotelemetry was significantly higher in *Gstm1* KO mice: WT 129.0±4.9 versus KO 135.6±2.7 mm Hg; P=0.02; n=6 each. (B) Urinary ACR was not different between groups: WT 27.3±7.2 versus KO 29.1±10.1 μg/mg; P=0.73; n=6 each. (C) Twenty four hour excretion of urinary 8-isoprostane was elevated in *Gstm1* KO mice: WT 6.6±1.2 versus KO 14.2±3.1 ng/100 mg body wt/24 h; P=0.001; n=5/6. All comparisons by t test. *P<0.05; **P<0.01.

suggesting that this axis plays a role in adaptive response after loss of nephron mass. We subjected eight WT and *Gstm1* KO mice to the Nx-CKD model. *Gstm1* KO mice had significantly worse survival after Nx, with a 23% survival rate at 83 days (12 weeks) after the procedure, whereas there was no mortality in WT mice at 90 days (13 weeks, original study end point) (Figure 2A).

Because of the observed high mortality beyond 8 weeks, another cohort of mice underwent Nx and animals and tissue samples were analyzed at 56 days (8 weeks) after Nx. By radiotelemetry, *Gstm1* KO mice had significantly greater SBP (Figure 2B), and elevated renal superoxide levels as measured by lucigenin luminescence (Figure 2C), consistent with the notion that GSTM1 plays a role in regulating oxidative stress. Gstm1 KO mice had higher urinary ACR (Figure 2D), and quantitation of kidney histopathology (Figure 2E, Supplemental Figure 2B) confirmed they had greater tissue injury, characterized by severe glomerulosclerosis and chronic inflammation. Digital quantitation of glomerular and mesangial surface area showed a significant increase in both measures in *Gstm1* KO mice (Figure 2F), suggesting that glomerular hyperfiltration and increased glomerular remodeling[39] is influenced by deletion of *GSTM1*.

Given the significance of the podocytes to the renal filtration apparatus and the increased albuminuria in *Gstm1* KO mice after Nx, we set out to determine whether GSTM1 deficiency has a direct effect on podocyte health. Primary podocytes (synaptopodin-positive cells) were isolated from healthy, unmanipulated WT and KO kidneys, and the GSTM1 expression was found only in WT cells, as expected (Supplemental Figure 2, C–E). Migration capacity using a standard *ex vivo* scratch assay[35] showed that *Gstm1* KO podocytes migrate at a significantly higher rate than those from WT mice, as reflected by the significantly smaller noncovered area after 14 hours (Figure 2G). The motile phenotype of podocytes *in vitro* is generally considered to be analogous to podocyte foot effacement and injury *in vivo*.[40,41] These data suggest that deletion of *Gstm1* may exaggerate podocyte response to stress and injury, and contribute to the increased glomerular injury observed in mice with Nx-CKD.

Deletion of *Gstm1* Results in Exaggerated Kidney Injury Mediated by Oxidative Stress in the AngII-HTN Model

To determine the generalizability of the effect of GSTM1 deficiency on susceptibility to kidney injury in disease states, we next determined the effect of *Gstm1* deletion in a model of HTN induced by AngII infusion. Despite a modest increase in baseline SBP in *Gstm1* KO mice, there was no difference in the severity of HTN (Figure 3A) or urinary ACR (Figure 3B). However, *Gstm1* KO mice had approximately three times higher superoxide radicals in kidney tissue (Figure 3C), despite a lack of difference in NADPH subunits (Nox2 and Nox4, Supplemental Figure 3A)[42] and SOD (cytosolic CuZn-SOD [SOD1], extracellular CuZn-SOD [SOD3], and mitochondrial Mn-SOD [SOD2], Supplemental Figure 3B). *Gstm1* KO mice also had greater tissue injury compared with WT mice (Figure 3D, Supplemental Figure 3C). We next quantified indicators of inflammation given its significance in the development of AngII-HTN,[43,44] and found that KO mice had a significant increase in renal expression of several genes involved in inflammation, including CXCL-1, MCP-1, and IL-6 (Figure 3E, Supplemental Figure 3D).

Because the AngII-HTN model had fewer surgery-related complications and mortality compared with the Nx-CKD model, further studies were conducted in the AngII-HTN

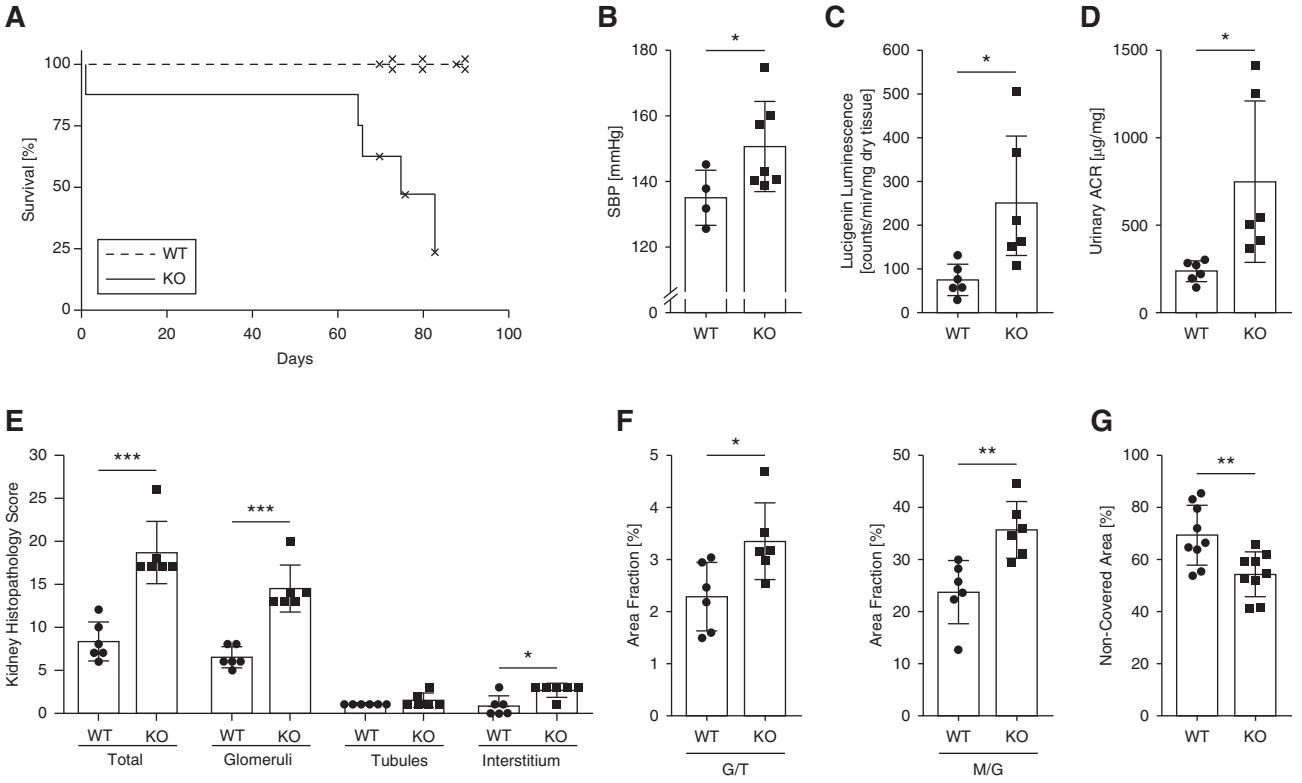

Figure 2. Deletion of *Gstm1* in the Nx CDK model results in poor survival and exaggerated HTN and kidney injury. (A) Survival curve showing significant mortality in *Gstm1* KO mice after Nx; P=0.01 versus WT; n=8 each. Time of censoring for mice that were used for terminal experiments are marked by ×. (B) SBP measured by radiotelemetry was significantly higher in *Gstm1* KO mice: WT 135.0±8.4 versus KO 150.6±13.7 mm Hg; P=0.04; n=4/7. (C) Renal superoxide levels measured by lucigenin luminescence and normalized to dry tissue weight were higher in *Gstm1* KO mice: WT 74.5±36.1 versus KO 250.4±153.6; P=0.04; n=6 each. (D) Urinary ACR was higher in *Gstm1* KO mice: WT 235.1±60.3 versus KO 748.4±460.4 µg/mg; P=0.04; n=6 each. (E) Kidney pathology scores were greater in *Gstm1* KO mice for all compartments (total: WT 8.3±2.3 versus KO 18.7±3.6; P<0.001) and in most individual compartments (glomeruli: WT 6.5±1.2 versus KO 14.5±2.7; P<0.001; tubules: WT 1.0±0.0 versus KO 1.5±0.8; P=0.20; interstitium: WT 0.8±1.2 versus KO 2.7±0.8; P=0.01), n=6 each. (F) Area fractions were greater in *Gstm1* KO mice for glomerular to total noninfarcted kidney (G/T, left: WT 2.3±0.7 versus KO 3.3±0.7; P=0.03) and mesangial to glomerular (M/G, right: WT 23.7±6.1 versus KO 35.7±5.5; P=0.005), n=6 each. (G) Percent of scratched area that remained uncovered after 14 hours was reduced in *Gstm1* KO mice: WT 69.3±11.5 versus KO 54.2±8.6; P=0.007; n=9 each. All comparisons by t test. *P<0.05; **P<0.01; ***P<0.001.

model. To determine whether oxidative stress was mediating the exaggerated disease phenotype in *Gstm1* KO mice, Tempol, a superoxide scavenger, was administered concurrently with AngII. Although Tempol caused a mild increase in SBP (Figure 3A) and no change in ACR (Figure 3B), it did significantly decrease renal superoxide levels (Figure 3C), kidney pathology (Figures 3D, Supplemental Figure 3C), and expression of MCP-1 and CXCL-1 (Figure 3E) in KO mice. Although it is unclear why SBP increased in KO mice treated with Tempol, these findings suggest that deletion of *Gstm1* enhances kidney injury through an oxidative stress pathway independent of BP.

Gstm1 KO Mice in the AngII-HTN Model Have Increased Renal Inflammation associated with the Deletion of *Gstm1* in the Parenchyma

Increased expression of chemokines and cytokines in *Gstm1* KO kidneys suggested increased renal inflammation. We next used flow cytometry to quantify in an unbiased manner the proportion of inflammatory cells in AngII-HTN WT and KO kidneys. The gating strategy is shown in Supplemental Figure 4A. Analysis showed more adaptive ($CD4^+$ and $CD8^+$ T cells) and innate (neutrophils [Ly6G, PMN] and macrophages [F4/80]) inflammatory cells in the *Gstm1* KO kidney (Figure 4A) that were primarily sequestered in the renal interstitium (Supplemental Figure 4, B and C). This suggests that during AngII-HTN, loss of GSTM1 augments renal inflammation.

Because GSTM1 is abundantly expressed in hematopoietic cells and renal epithelial cells, and CXCL-1 and MCP-1 are not uniquely expressed in macrophages and neutrophils but are also expressed in renal epithelial cells, we next investigated whether deletion of *Gstm1* in BM-derived cells or in the parenchyma contributes to renal inflammation and injury in the AngII-HTN model, using a BM crosstransplant approach. Analysis of the renal $CD45^+$ population by two-way ANOVA

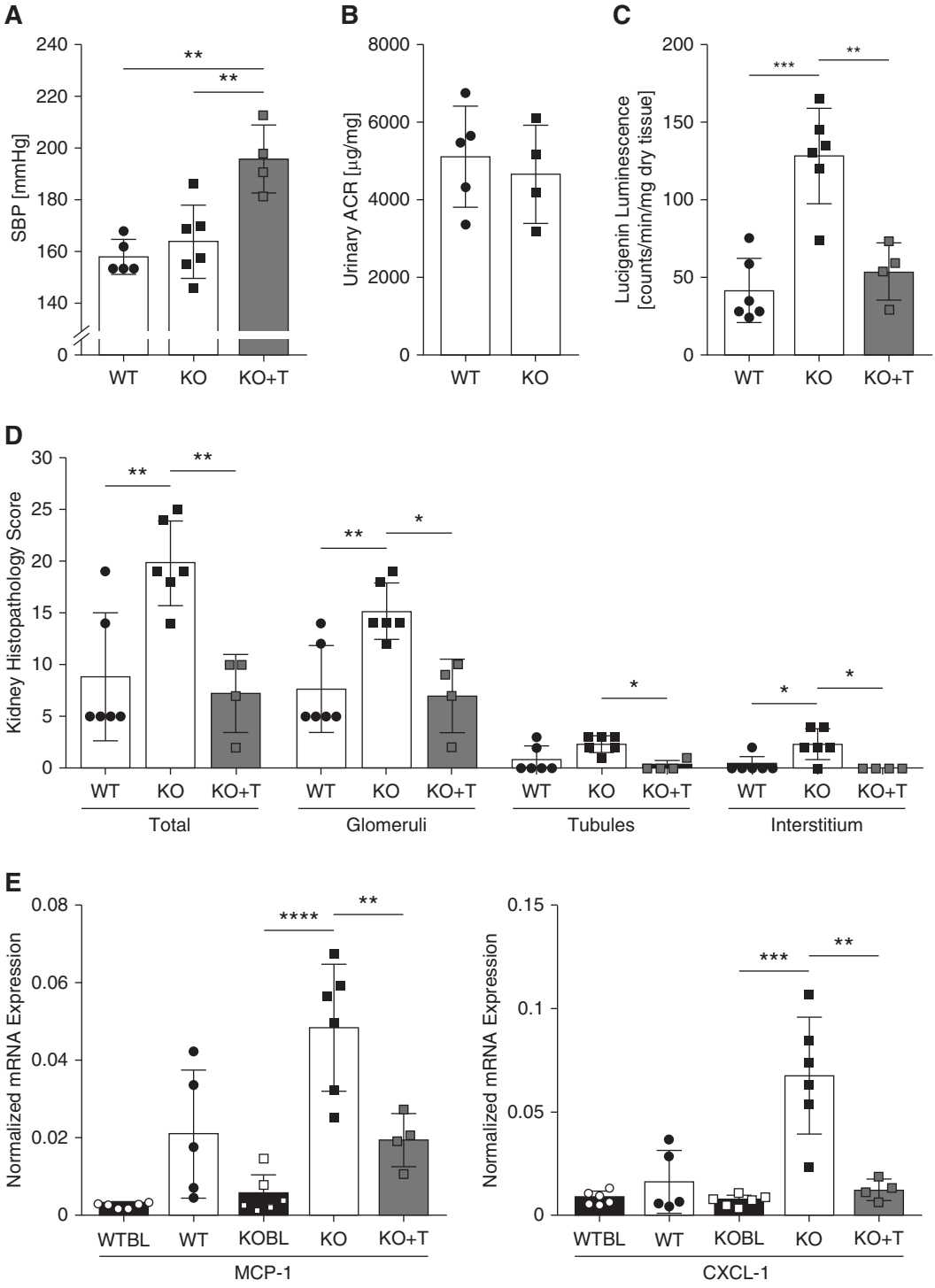

Figure 3. Deletion of *Gstm1* in the AngII HTN model results in exaggerated oxidative stress and kidney injury that is ameliorated by Tempol. (A) SBP measured by radiotelemetry was significantly higher in *Gstm1* KO mice treated with Tempol (KO+T): WT 157.8±6.7 versus KO 163.7±14.1 versus KO+T 195.6±13.2 mm Hg; P=0.001; n=5/6/4. (B) Urinary ACR was not significantly different: WT 5108.2±1302.9 versus KO 4657.9±1262.2 μg/mg; P=0.62; n=5/4. (C) Renal superoxide levels measured by lucigenin luminescence and normalized to dry tissue weight were higher in *Gstm1* KO mice: WT 41.6±20.8 versus KO 128.25±30.5 versus KO+T 53.8±18.4; $P<0.001$; n=6/6/4. (D) Kidney pathology scores were greater in *Gstm1* KO mice for all compartments (total: WT 8.8±6.1 versus KO 19.8±4.1 versus KO+T 7.3±3.8; P=0.002) and in individual compartments (glomeruli: WT 7.7±4.2 versus KO 15.2±2.7 versus KO+T 7.0±3.6; P=0.003; tubules: WT 0.8±1.3 versus KO 2.3±0.8 versus KO+T 0.3±0.5; P=0.01; interstitium: WT 0.3±0.8 versus KO 2.3±1.5 versus KO+T 0.0±0.0; P=0.006), n=6/6/4. (E) Renal mRNA levels of genes involved in inflammation normalized to *Hprt* in WT mice at

revealed that the recipient genotype significantly influenced the degree of renal inflammation, with KO recipient mice having increased renal inflammation. There was no significant effect of donor genotype nor of the interaction between donor and recipient. Hence, our study shows that deletion of *Gstm1* in the parenchyma, and not in BM-derived cells, drives renal inflammation and injury.

Supplementation with SRBP Ameliorates Kidney Injury in *Gstm1* KO Mice but Not in WT Mice

We first confirmed the bioavailability and activity of synthetic sulforaphane (Supplemental Figure 5A) and showed that dietary supplementation of SRBP produced similar effects in WT mice without effect on body weight (Supplemental Figure 5, B and C). As expected, SRBP induced *Gstm1* expression in the kidney in WT mice during AngII-HTN, but not in KO mice in which *Gstm1* expression was not detectable (Supplemental Figure 5D). SRBP supplementation did not lower SBP in either WT or KO mice in the AngII-HTN model (Figure 5A), and in fact increased SBP in WT mice. However, SRBP significantly decreased renal levels of superoxide (Figure 5B), ACR (Figure 5C), and kidney histopathology scores in the glomeruli and interstitium in KO mice (Figure 5D, Supplemental Figure 5E). In most of these metrics, KO mice fed SRBP were similar to WT mice fed SRBP. In contrast, WT mice treated with SRBP had no significant change compared with untreated WT mice. Our result suggests that the beneficial effect of SRBP is dependent on the absence *Gstm1*.

In Humans, *GSTM1* Deletion Modifies the Protective Effect of Cruciferous Vegetables Against Kidney Failure

To determine whether our findings of the protective effect of SRBP in *GSTM1* KO mice can be translated to humans, we evaluated whether *GSTM1* deletion modified the association between cruciferous vegetable intake and kidney failure in the ARIC study.

Population Characteristics

Among 10,155 participants in the ARIC study, the mean age was 54 years, 44% were men, and 27% were black. The mean baseline eGFR was 103 ml/min per 1.73 m^2. The participants in the three cruciferous vegetable intake categories did not differ significantly by age, body mass index, prevalent diabetes, or baseline eGFR. By the three cruciferous vegetable intake categories, the high-intake group had significantly lower proportion of men (high intake: 32.6%, low intake: 57.0%), black participants (high intake: 17.5%, low intake: 34.7%), and current smokers (high intake: 22.3%, low intake 30.9%) (Table 1). Among the participants in this study, a subsample (n=5461) was previously studied for the association between *GSTM1* deletion and kidney failure.[3] Compared with the previous subsample, the additional participants in this study (n=4694) had higher intake of cruciferous vegetables (high intake: previous subsample 22.0%, additional participants 26.7%; $P<0.001$; Supplemental Table 1).

The frequencies of *GSTM1* deletion genotype among black and white participants were similar to the race-specific frequencies reported in other study populations (black participants: *GSTM1(0/0)*, 26.1%; *GSTM1(0/1)*, 48.3%; *GSTM1(1/1)*, 25.7%; white participants *GSTM1(0/0)*, 52.3%; *GSTM1(0/1)*, 39.5%; *GSTM1(1/1)*, 8.2%; Supplemental Table 2).[4]

Protective Association of Higher Cruciferous Vegetable Intake was Mainly Observed among Participants with GSTM1 Homozygous Deletion

Over a median of 24.6 years, 370 kidney failure events were observed (black participants, 188; white participants, 182). In the primary analysis, the protective association of higher intake of cruciferous vegetable for kidney failure was significantly stronger among those with *GSTM1(0/0)* genotype than among those with *GSTM1(0/1) or GSTM1(1/1)* genotypes (P value for interaction between cruciferous vegetable intake and *GSTM1* genotype <0.05). Among participants with *GSTM1(0/0)* genotype, in model 1, higher intake of cruciferous vegetables was associated with >40% reduction in risk of kidney failure (model 1 race-combined hazard ratio [HR]: medium intake, 0.58; 95% confidence interval [95% CI], 0.41 to 0.82; high intake, 0.41; 95% CI, 0.25 to 0.68; P-trend<0.001; Figure 6, Table 2). With the additional adjustment for clinical risk factors, this association remained similar (model 2 race-combined HR: medium intake, 0.68; 95% CI, 0.48 to 0.98; high intake, 0.49; 95% CI, 0.29 to 0.83; P-trend=0.005). Among those with *GSTM1(0/1) or GSTM1(1/1)* genotypes, cruciferous vegetable intake did not have significant dose response association with kidney failure (model 1: race-combined P-trend=0.45; model 2: race-combined P-trend=0.46; P value for interaction between cruciferous vegetable intake and *GSTM1* genotype, Model 1: 0.02; Model 2: 0.03) (Table 2). Analysis with *GSTM1(0/1) and GSTM1(1/1)* separately yielded similar results. (Supplemental Table 3).

In the analysis within each race group, the association between cruciferous vegetable intake and kidney failure by *GSTM1* genotype was consistent across the two race groups (all P for interaction between race and cruciferous vegetable intake within *GSTM1* strata >0.05; Supplemental Table 4). These results suggest *GSTM1* deletion modifies the protective effect of cruciferous vegetable for kidney failure across populations.

baseline (WTBL) and with HTN (WT), n=6/5 and KO mice at baseline (KOBL), with HTN (KO) and with HTN treated with Tempol (KO+T), n=6/6/4. Expression levels were elevated in the KO mice for MCP-1 (KOBL 0.0054±0.0050 versus KO 0.048±0.016 versus KO+T 0.019±0.0068; $P<0.001$) and CXCL-1 (KOBL 0.0071±0.0027 versus KO 0.068±0.028 versus KO+T 0.012±0.0051; $P<0.001$). WT comparisons in (E) by t test. Comparisons in other panels and KO comparisons in (E) by one-way ANOVA. *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.001$.

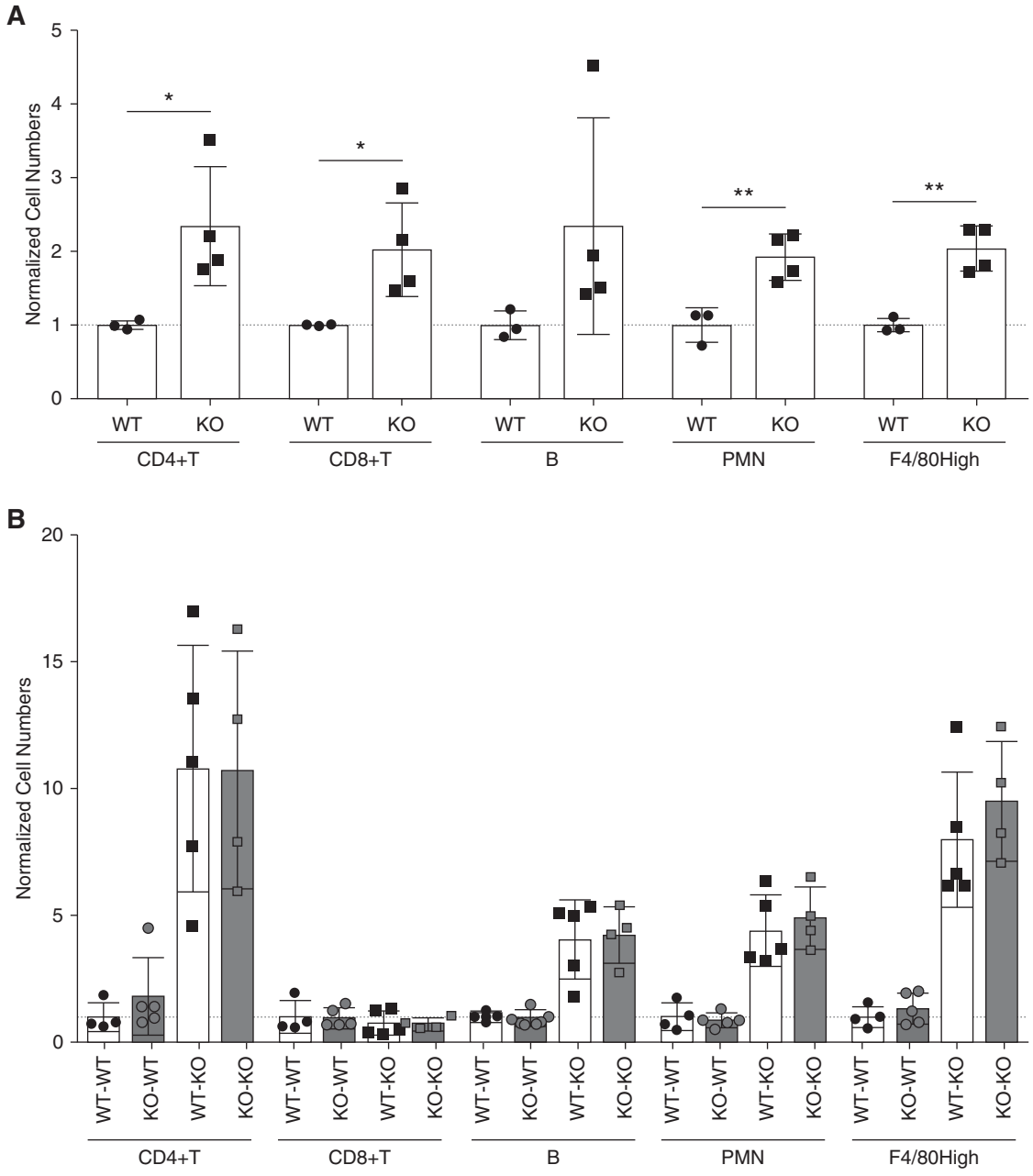

Figure 4. *Gstm1* KO mice in the AngII HTN model have increased renal inflammation associated with the deletion of *Gstm1* in the parenchyma. (A) Flow cytometry data of renal leukocytes normalized to WT counts showed *Gstm1* KO mice have increased populations (*P* values: CD4+T=0.04; CD8+T=0.05; B=0.16; PMN=0.007; $F4/80_{High}$=0.004), *n*=3/4. Comparisons by one-way ANOVA. **P*<0.05; ***P*<0.01. (B) Flow cytometry data of renal leukocytes for BM chimeras generated from BM crosstransplantation (genotypes listed as donor-recipient) normalized to WT-WT showed recipient genotype was the statistically significant factor (donor *P* values: CD4+T=0.82; CD8+T=0.95; B=0.87; PMN=0.70; $F4/80_{High}$=0.31; recipient *P* values: CD4+T=5.9×10^{-5}; CD8+T=0.30; B=1.1×10^{-5}; PMN=1.5×10^{-6}; $F4/80_{High}$=5.0×10^{-7}), *n*=4/5/5/4. Comparisons by two-way ANOVA.

Association between GSTM1 Deletion and Kidney Failure by Cruciferous Vegetable Intake Levels

In the analysis comparing *GSTM1(0/0)* versus *GSTM1(0/1)* or *GSTM1(1/1)*, *GSTM1(0/0)* did not have significant association with kidney failure within each of the cruciferous intake category adjusting for demographic and clinical covariates (Supplemental Table 5). When the three *GSTM1* deletion genotypes were analyzed separately, *GSTM1* deletion genotypes (*0/0* and *0/1*) were associated with higher risk for kidney failure compared with *GSTM1(1/1)* among those with medium intake (*P*-trend=0.03; Supplemental Table 6).

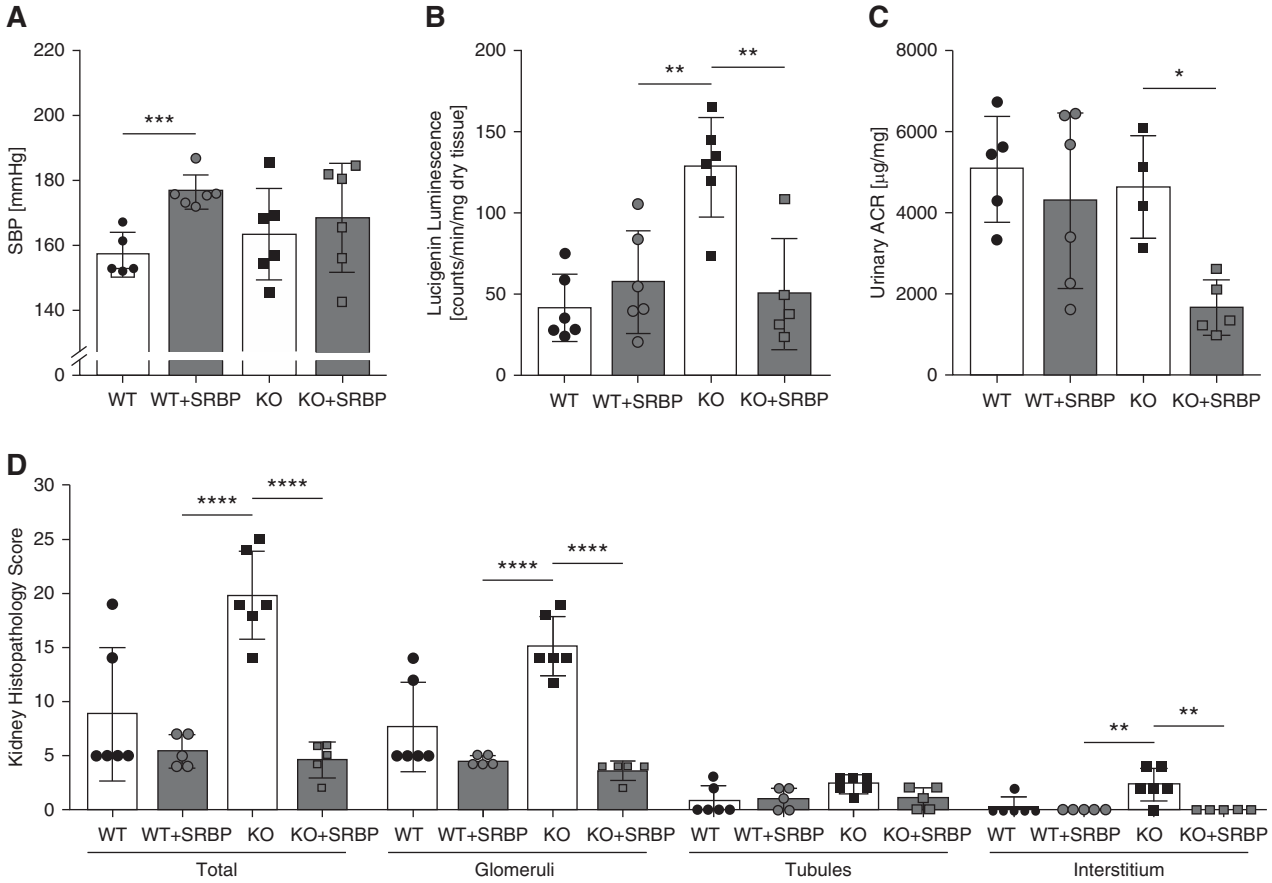

Figure 5. Treatment with SRBP ameliorated kidney injury only in *Gstm1* KO mice. In all panels, WT and KO mice are the same as those in Figure 3, with the data reproduced here for ease of comparison. (A) SBP measured by radiotelemetry was elevated in WT mice fed SRBP (WT+SRBP 176.9±5.9 mm Hg; P=0.001; n=6) but not in *Gstm1* KO mice fed SRBP (KO+SRBP 168.9±3.5 mm Hg; P=0.24; n=6). (B) Renal superoxide levels measured by lucigenin luminescence and normalized to dry tissue weight were not affected by SRBP in WT mice (WT+SRBP 57.7±31.5 counts/min per milligram dry tissue; P=0.02; n=6) but were reduced in *Gstm1* KO mice (KO+SRBP 50.4±33.9 counts/min per milligram dry tissue; *post hoc* versus KO P=0.004; n=5). (C) Urinary ACR was reduced by SRBP in *Gstm1* KO mice (KO+SRBP 1671.5±683.6 µg/mg; *post hoc* versus KO P=0.05; n=5), but not WT mice (WT+SRBP 4314.4±2155.3 µg/mg; P=0.47; n=6). (D) Kidney pathology scores were unaffected by SRBP in WT mice (total: 5.4±1.5; P=0.24; glomeruli: 4.4±0.5; P=0.11; tubules: 1.0±1.0; P=0.82; interstitium: 0.0±0.0; P=0.36; n=5) but were reduced in *Gstm1* KO mice (total: 4.6±1.7; *post hoc* versus KO $P=2.0\times10^{-6}$; glomeruli: 3.6±0.9; *post hoc* versus KO $P=2.4\times10^{-7}$; tubules: 1.0±1.0; *post hoc* versus KO P=0.10; interstitium: 0.0±0.0; P=0.004; n=5). Comparisons between WT and WT+SRBP by *t* test. Comparisons between WT+SRBP, KO, and KO+SRBP by one-way ANOVA. *P<0.05; **P<0.01; ***P<0.001; ****P<0.001.

Overall Association of Cruciferous Vegetable Intake and GSTM1 Deletion with Kidney Failure

Overall higher cruciferous vegetable intake was associated with lower risk of kidney failure (model 2 race-combined HR: medium intake, 0.68; 95% CI, 0.53 to 0.86; high intake, 0.70; 95% CI, 0.51 to 0.96; P-trend=0.01) (Supplemental Table 7). Similar results were observed within the race groups. In contrast, *GSTM1(0/0)* did not have significant overall association with kidney failure when compared with *GSTM1(0/1)* and *GSTM1(1/1)* combined (Supplemental Table 8) or by the three deletion genotypes (model 2 race-combined HR: *GSTM1(0/0)*, 1.30; 95% CI, 0.92 to 1.82; *GSTM1(0/1)*, 1.24; 95% CI, 0.89 to 1.72; P-trend=0.18; Supplemental Table 9), suggesting that the main effect of *GSTM1* deletion can be highly dependent on environmental factors.

DISCUSSION

We have shown that *Gstm1* deletion in the mouse directly influences the severity of kidney injury in two disease models (Nx-CKD and AngII-HTN). In the Nx-CKD model, *Gstm1* deletion resulted in poor survival and exaggerated HTN and kidney injury. In the AngII-HTN model, *Gstm1* deletion resulted in exaggerated kidney injury associated with heightened renal inflammation. This is likely mediated by oxidative stress, which was increased in both models, as treatment with

Table 1. Baseline population characteristics by cruciferous vegetable intake category (n=10, 155)

Characteristic	Low Intake	Medium Intake	High Intake	*P* Value
N	2017	5680	2458	
Age, yr, mean (SD)	54.4 (5.8)	54.6 (5.7)	54.4 (5.6)	0.29
Men, *n* (%)	1150 (57.0)	2512 (44.2)	801 (32.6)	<0.001
Black, *n* (%)	700 (34.7)	1582 (27.9)	431 (17.5)	<0.001
Body mass index, kg/m^2, mean (SD)	27.57 (5.28)	27.78 (5.3)	27.65 (5.48)	0.25
Smoking, *n* (%)				<0.001
Current smoker	623 (30.9)	1412 (24.9)	548 (22.3)	
Former smoker	670 (33.2)	1856 (32.7)	806 (32.8)	
Never smoked	724 (35.9)	2412 (42.5)	1104 (44.9)	
Diabetes, *n* (%)	222 (11)	645 (11.4)	258 (10.5)	0.52
HTN, *n* (%)	741 (36.7)	1990 (35)	762 (31)	<0.001
Coronary heart disease, *n* (%)	114 (5.7)	282 (5)	105 (4.3)	0.10
eGFR, ml/min per 1.73 m^2, mean (SD)	103.31 (16.38)	102.75 (15.51)	102.25 (14.19)	0.07
Education, *n* (%)				<0.001
Less than high school	595 (29.5)	1154 (20.3)	360 (14.6)	
Some college	817 (40.5)	2358 (41.5)	1015 (41.3)	
Graduate education	605 (30)	2168 (38.2)	1083 (44.1)	
Physical activity score, median (25 percentile, 75 percentile)	2.2 (1.8, 2.5)	2.2 (2, 2.8)	2.5 (2.2, 2.8)	<0.001
Total calorie intake, median (25 percentile, 75 percentile)	1475 (1133, 1913)	1511 (1177, 1918)	1633 (1283, 2056)	<0.001

Intake categories: low intake, three or more times per month; medium, more than three times per month but less than once per week; high intake, at least once per week. *P* values for baseline characteristics of participants by three levels of cruciferous vegetable intake were obtained using *t* tests for nonskewed continuous variables, Wilcoxon tests for skewed continuous variables, and chi-squared tests for categorical variables.

Tempol, a superoxide scavenger, in the AngII-HTN model reduced oxidative stress, expression of MCP-1 and CXCL-1, and kidney injury without lowering BP in *Gstm1* KO mice. BM crosstransplantation delineated that *Gstm1* in the parenchyma, and not in the hematopoietic cells, determined renal inflammation. Finally, *Gstm1* deletion modified the effect of SRBP supplementation, as SRBP supplementation reduced superoxide levels, reduced ACR, and improved kidney histopathology scores in KO mice but not in WT. This modifying effect of *GSTM1* deletion in mice on SRBP supplementation was also observed in humans in the ARIC study, where the protective effect of cruciferous vegetable intake on kidney failure was mainly found in those with homozygous deletion of *GSTM1*.

Gstm1 is a positional candidate gene for HTN in the SHRSP rats.[31] Here, we found that deletion of *Gstm1* in the mouse resulted in a small but significant increase in SBP at baseline

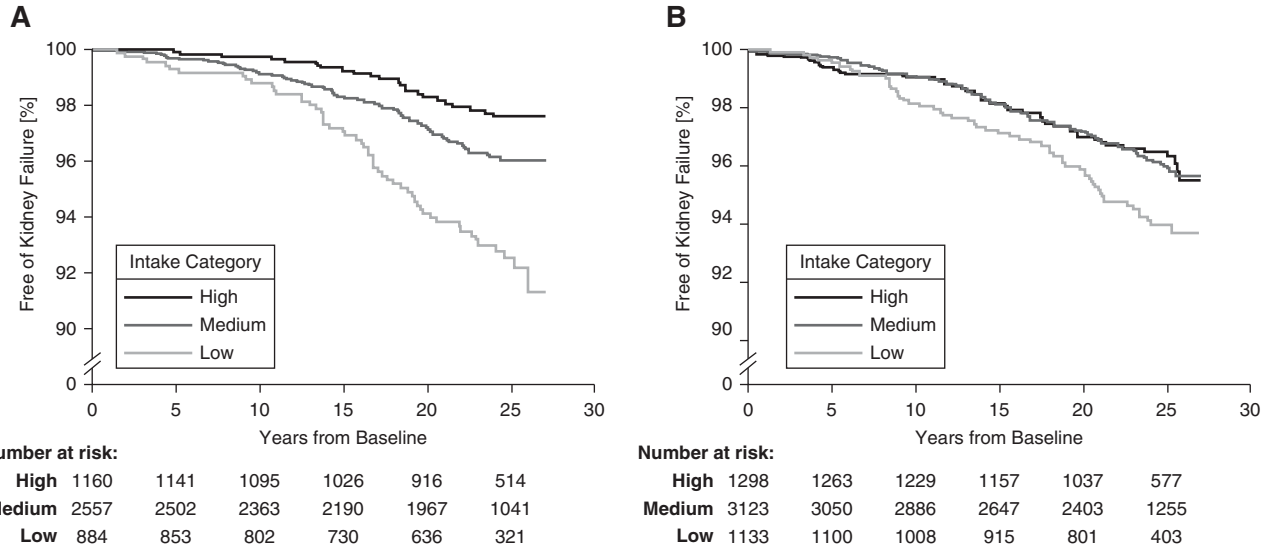

Figure 6. Humans with *GSTM1(0/0)* have reduced incidence of kidney failure with higher cruciferous vegetable intake. Kaplan–Meier plots of kidney failure by cruciferous vegetable intake category within those (A) with and (B) without *GSTM1* homozygous deletion showed a significant benefit only in those with the homozygous deletion. See Table 2 for statistical analysis.

Table 2. Association between cruciferous vegetable intake and kidney failure stratified by *GSTM1*

	HR (95% CI)			
	Low Intake	Medium Intake	High Intake	*P*-Trend
Model 1				
GSTM1 (0/0)	Reference	0.58 (0.41 to 0.82)	0.41 (0.25 to 0.68)	<0.001
GSTM1 (0/1 or 1/1)	Reference	0.73 (0.53 to 1.01)	0.88 (0.59 to 1.33)	0.45
Model 2				
GSTM1 (0/0)	Reference	0.68 (0.48 to 0.98)	0.49 (0.29 to 0.83)	0.005
GSTM1 (0/1 or 1/1)	Reference	0.68 (0.49 to 0.94)	0.89 (0.59 to 1.36)	0.46

n (event). GSTM1(0/0), 4601 (159); GSTM1(0/1 or 1/1), 5554 (211).
Model 1 covariates: age, sex, race, center, genetic principal components. Model 2 covariates: model 1 plus eGFR, prevalent diabetes, HTN, coronary heart disease, smoking status, body mass index, leisure physical activity, education levels, and total calorie intake. *P*-trend was obtained by using cruciferous vegetable intake as a continuous variable in Cox regression. *P* value for *GSTM1* and cruciferous vegetable intake interaction: model 1=0.02; model 2=0.03.

and exaggerated HTN in the Nx-CKD model. This could be explained by increased oxidative stress at baseline and more severe kidney injury in the Nx-CKD model. Our result is consistent with a recent study showing that transgenic overexpression of *Gstm1* in the kidney injury-prone SHRSP rat rescued renal oxidative stress and HTN.[45] In the AngII-HTN model, there was no difference in SBP between WT and KO mice and we speculate that the very high dose of AngII administered potentially masked any modest BP effect of *Gstm1* deletion.

In both Nx-CKD and AngII-HTN induced disease models, we demonstrated that deletion of *Gstm1* directly influenced the severity of kidney injury in the mouse and that this was associated with increased renal oxidative stress. Although we cannot at this time attribute worse kidney injury to worse HTN or *vice versa* in the Nx-CKD model, these two phenotypes in *Gstm1* KO mice correlate with those seen in human patients with CKD in whom HTN becomes more severe with worsening kidney disease.[46]

The mechanism(s) by which *Gstm1* deletion exaggerates kidney disease is likely through oxidative stress and inflammation, as both Tempol and SRBP lowered renal superoxide levels, inflammation, and kidney pathology scores, without lowering BP, in the AngII-HTN model. We previously reported that knockdown of *Gstm1* in VSMCs *in vitro* resulted in increased levels of superoxide.[29] Our present *in vivo* studies found that deletion of *Gstm1* also resulted in increased renal superoxide in both the Nx-CKD and AngII-HTN models. There is no evidence that GSTM1 has SOD activity to metabolize superoxide. Moreover, deletion of *Gstm1* did not result in increased expression of Nox2 or Nox4 isoforms of NAD(P)H oxidase, nor altered expression level of SOD in the AngII-HTN model. These data suggest that GSTM1 might influence superoxide metabolism indirectly. Because GSTM1 enzyme has overlapping substrate specificities with other GST enzymes, the increased superoxide levels in *Gstm1* KO mice also suggests that there is inadequate compensation by other GST enzyme(s) or pathway(s) that determine superoxide levels. Furthermore, enzyme kinetic assays have demonstrated that GSTM1 has activity against epoxides[47] and several reactive aldehydes[48–50] and, *in vivo*, transgenic overexpression of *Gstm1* in the SHRSP rat decreases renal levels of malondialdehyde.[45] The multiple substrates metabolized by GSTM1 suggest that their metabolites and downstream effects may be influenced by disease states. Identification of reactive oxygen species regulated by GSTM1 that are involved in kidney injury in these models would be informative to further elucidate the mechanisms.

In the Nx-CKD model, *Gstm1* KO mice displayed increased mortality, in addition to worse HTN, oxidative stress, and kidney injury. The reason for the poor survival of these mice beyond 8 weeks after Nx is unclear. The increased ACR and glomerular and mesangial surface areas in *Gstm1* KO mice may reflect increased glomerular HTN from GSTM1 deficiency, raising the question whether GSTM1 deficiency influences single nephron GFR, preglomerular myogenic tone, and postglomerular vasodilation. Furthermore, we report for the first time that GSTM1 is normally expressed in podocytes, and that GSTM1 deficiency increases podocyte migration in response to wound scratch *in vitro.* The "motile" phenotype of podocytes *in vitro* is considered analogous to podocyte injury and effacement *in vivo*.[40,51] The potential role of GSTM1 in glomerular hyperfiltration and podocyte function in disease states will be a focus of future studies.

In the AngII-HTN model, deletion of *Gstm1* resulted in worse kidney injury, but not an increase in mortality as in the Nx-CKD model. Thus, further experiments were all conducted using the AngII-HTN model. The kidney injury in the AngII-HTN model was associated with increased expression of genes involved in inflammation, particularly those expressed in macrophages, raising the possibility that GSTM1 may mediate macrophage inflammatory response in certain disease states. This is consistent with findings by Alexis *et al.*,[52] who reported that human volunteers with the *GSTM1* null genotype exposed to ozone had significantly increased expression of HLA-DR on airway macrophages and dendritic cells. Furthermore, SRBP inhibited MIF tautomerase, and decreased renal inflammation in *Gstm1* KO mice. CXCL-1 and MCP-1 are not uniquely expressed in macrophages and neutrophils, but are also expressed in endothelial[53] and renal epithelial cells.[54] Our BM chimeras suggest that deletion of *Gstm1* determines renal inflammation in AngII-HTN mechanistically through a parenchymal-driven pathway rather than *via* the hematopoietic cells. However, we cannot rule out that *Gstm1* deletion activates resident leukocytes in the kidney using the BM crosstransplantation approach.

Although deletion of *Gstm1* increased kidney injury and inflammation, dietary administration of SRBP in the mice protected against renal injury and inflammation only in

Gstm1 KO mice. Similarly, the protective effect of cruciferous vegetable consumption in lowering the risk of kidney failure in ARIC participants was observed largely in those with *GSTM1(0/0)* genotype. This modifying effect of *GSTM1* is consistent with that observed in lung cancer, illustrating the pleiotropic effect of *GSTM1*. We have shown that *GSTM1* deletion modifies the effect of the renal risk variants in the apoL1 (*APOL1)* gene,[55] suggesting that the modifying effects of this deletion may extend even further. It is worth noting that *APOL1* genotype in the donor kidney, and not in the recipient, determines outcomes in kidney transplantation.[56] This raises the question how APOL1 and GSTM1 interact in the kidney to affect susceptibility to kidney injury and kidney disease progression.

Taken together, these studies suggest that *GSTM1* deletion may be a modifier of a broad range of disease phenotypes. The lack of phenotype in KO mice at 1 year of age is consistent with the notion that GSTM1 deletion is a modifier of stressors. We speculate that loss of GSTM1 enzyme can increase the accumulation of toxic metabolites and reactive oxygen species, thereby worsening disease progression; however, GSTM1 deficiency can also increase the level of protective metabolites from dietary intake in disease states, such as with sulforaphane, which activates the Nrf2 signaling pathway, thereby inducing a wide variety of phase 2 detoxification enzymes.[23,24] In line with this notion, in the Sulforaphane in Treating Patients with Recurrent Prostate Cancer Trial, those with the *GSMT1* null genotype have a longer median half-life ($t_{1/2}$) of sulforaphane in the blood (2.6, range [2.0–5.5] hours), compared with those with the active genotype (2.1, range [1.8–2.9] hours) (https://clinicaltrials.gov/ct2/show/results/NCT01228084?sect=Xedc0156#outcome8). Another study reported that bioavailability of free sulforaphane and sulforaphane metabolites were increased in *GSTM1* null subjects compared with those with the active allele.[25] Moreover, we show in the mouse that SRBP can increase the expression level of *GSTM1* in WT mice, which may in turn further decrease the bioavailability of sulforaphane, thereby limiting its beneficial effect of activating other antioxidants in kidney disease in WT mice. Furthermore, upregulation of phase 2 antioxidants by sulforaphane could explain its effectiveness in KO mice because there is increased ROS to scavenge even at baseline (Figure 1B). Delineation of the substrates/molecules in the sulforaphane-GSTM1 axis in kidney injury will be a focus of future studies.

It should be noted that in this study, the directions of association of *GSTM1* deletion with kidney failure were consistent with the previous smaller study[3] in ARIC, whereas the current effect sizes were smaller and not statistically significant. The association between *GSTM1* deletion and kidney failure in humans may depend on the levels of protective and toxic intracellular electrophiles that are ligands of *GSTM1*. The levels of these electrophiles vary by environment and study populations and thus the main effect of *GSTM1* deletion may vary across populations.

Our data support a dual role of GSTM enzyme in the modulation of oxidative stress, inflammation, and protective metabolites in kidney disease. Given the high prevalence of the *GSTM1* deletion, its population-attributable risk fractions on kidney function decline and ESRD was estimated to be sizeable at 38.4% in hypertensive black Americans.[55] Moreover, the risk is not race-specific and has similar effects in white Americans.[3] In the context of personalized and precision medicine, increased consumption of cruciferous vegetables and/or sulforaphane may be protective in those lacking *GSTM1* who are genetically most at risk.

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DISCLOSURES

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SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2019050449/-/DCSupplemental.

Supplemental Appendix 1. Supplemental methods and references.

Supplemental Figures 1–6 and legends.

Supplemental Tables 1–12.

REFERENCES

1. United States Renal Data System: *2017 USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States*, Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2017
2. Chang J, Ma JZ, Zeng Q, Cechova S, Gantz A, Nievergelt C, et al.: Loss of GSTM1, a NRF2 target, is associated with accelerated progression of hypertensive kidney disease in the African American Study of Kidney Disease (AASK). *Am J Physiol Renal Physiol* 304: F348–F355, 2013
3. Tin A, Scharpf R, Estrella MM, Yu B, Grove ML, Chang PP, et al.: The loss of *GSTM1* associates with kidney failure and heart failure. *J Am Soc Nephrol* 28: 3345–3352, 2017
4. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, et al.: Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 10: 1239–1248, 2001
5. Sprenger R, Schlagenhaufer R, Kerb R, Bruhn C, Brockmöller J, Roots I, et al.: Characterization of the glutathione S-transferase GSTT1 deletion: Discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype-phenotype correlation. *Pharmacogenetics* 10: 557–565, 2000
6. Xu S, Wang Y, Roe B, Pearson WR: Characterization of the human class Mu glutathione S-transferase gene cluster and the GSTM1 deletion. *J Biol Chem* 273: 3517–3527, 1998
7. Mannervik B, Board PG, Hayes JD, Listowsky I, Pearson WR: Nomenclature for mammalian soluble glutathione transferases. *Methods Enzymol* 401: 1–8, 2005
8. Hayes JD, Flanagan JU, Jowsey IR: Glutathione transferases. *Annu Rev Pharmacol Toxicol* 45: 51–88, 2005
9. Cho SG, Lee YH, Park HS, Ryoo K, Kang KW, Park J, et al.: Glutathione S-transferase mu modulates the stress-activated signals by suppressing apoptosis signal-regulating kinase 1. *J Biol Chem* 276: 12749–12755, 2001
10. Cotton SC, Sharp L, Little J, Brockton N: Glutathione S-transferase polymorphisms and colorectal cancer: A HuGE review. *Am J Epidemiol* 151: 7–32, 2000
11. Hou S-M, Ryberg D, Fält S, Deverill A, Tefre T, Børresen A-L, et al.: GSTM1 and NAT2 polymorphisms in operable and non-operable lung cancer patients. *Carcinogenesis* 21: 49–54, 2000
12. Capoluongo E, Onder G, Concolino P, Russo A, Santonocito C, Bernabei R, et al.: GSTM1-null polymorphism as possible risk marker for hypertension: Results from the aging and longevity study in the Sirente Geographic Area (iISIRENTE study). *Clin Chim Acta* 399: 92–96, 2009
13. Cruz-Gonzalez I, Corral E, Sanchez-Ledesma M, Sanchez-Rodriguez A, Martin-Luengo C, Gonzalez-Sarmiento R: An association between resistant hypertension and the null GSTM1 genotype. *J Hum Hypertens* 23: 556–558, 2009
14. Wang XL, Greco M, Sim AS, Duarte N, Wang J, Wilcken DE: Glutathione S-transferase mu1 deficiency, cigarette smoking and coronary artery disease. *J Cardiovasc Risk* 9: 25–31, 2002
15. Manfredi S, Federici C, Picano E, Botto N, Rizza A, Andreassi MG: GSTM1, GSTT1 and CYP1A1 detoxification gene polymorphisms and susceptibility to smoking-related coronary artery disease: A case-only study. *Mutat Res* 621: 106–112, 2007
16. Moon KS, Lee HJ, Hong SH, Kim HM, Um JY: CYP1A1 and GSTM1/T1 genetic variation in predicting risk for cerebral infarction. *J Mol Neurosci* 32: 155–159, 2007
17. Türkanoğlu A, Can Demirdöğen B, Demirkaya S, Bek S, Adali O: Association analysis of GSTT1, GSTM1 genotype polymorphisms and serum total GST activity with ischemic stroke risk. *Neurol Sci* 31: 727–734, 2010
18. Carpenter CL, Yu MC, London SJ: Dietary isothiocyanates, glutathione S-transferase M1 (GSTM1), and lung cancer risk in African Americans and Caucasians from Los Angeles County, California. *Nutr Cancer* 61: 492–499, 2009
19. Wang LI, Giovannucci EL, Hunter D, Neuberg D, Su L, Christiani DC: Dietary intake of cruciferous vegetables, glutathione S-transferase (GST) polymorphisms and lung cancer risk in a Caucasian population. *Cancer Causes Control* 15: 977–985, 2004
20. Zhao B, Seow A, Lee EJ, Poh WT, Teh M, Eng P, et al.: Dietary isothiocyanates, glutathione S-transferase -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol Biomarkers Prev* 10: 1063–1067, 2001
21. London SJ, Yuan JM, Chung FL, Gao YT, Coetzee GA, Ross RK, et al.: Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: A prospective study of men in Shanghai, China. *Lancet* 356: 724–729, 2000
22. Brennan P, Hsu CC, Moullan N, Szeszenia-Dabrowska N, Lissowska J, Zaridze D, et al.: Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: A mendelian randomisation approach. *Lancet* 366: 1558–1560, 2005
23. James D, Devaraj S, Bellur P, Lakkanna S, Vicini J, Boddupalli S: Novel concepts of broccoli sulforaphanes and disease: Induction of phase II antioxidant and detoxification enzymes by enhanced-glucoraphanin broccoli. *Nutr Rev* 70: 654–665, 2012
24. Boddupalli S, Mein JR, Lakkanna S, James DR: Induction of phase 2 antioxidant enzymes by broccoli sulforaphane: Perspectives in maintaining the antioxidant activity of vitamins A, C, and E. *Front Genet* 3: 7, 2012
25. Gasper AV, Al-Janobi A, Smith JA, Bacon JR, Fortun P, Atherton C, et al.: Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. *Am J Clin Nutr* 82: 1283–1291, 2005
26. Amin A, CanGongora M, Elbarbry F: Dietary doses of sulforaphane affect hepatic drug metabolizing enzymes in spontaneously hypertensive rats. *Phytother Res* 29: 1412–1420, 2015
27. Elbarbry F, Vermehren-Schmaedick A, Balkowiec A: Modulation of arachidonic acid metabolism in the rat kidney by sulforaphane: Implications for regulation of blood pressure. *ISRN Pharmacol* 2014: 683508, 2014
28. Le TH, Fogo AB, Salzler HR, Vinogradova T, Oliverio MI, Marchuk DA, et al.: Modifier locus on mouse chromosome 3 for renal vascular pathology in AT1A receptor-deficiency. *Hypertension* 43: 445–451, 2004
29. Yang Y, Parsons KK, Chi L, Malakauskas SM, Le TH: Glutathione S-transferase-micro1 regulates vascular smooth muscle cell proliferation, migration, and oxidative stress. *Hypertension* 54: 1360–1368, 2009
30. McBride MW, Carr FJ, Graham D, Anderson NH, Clark JS, Lee WK, et al.: Microarray analysis of rat chromosome 2 congenic strains. *Hypertension* 41: 847–853, 2003
31. McBride MW, Brosnan MJ, Mathers J, McLellan LI, Miller WH, Graham D, et al.: Reduction of Gstm1 expression in the stroke-prone spontaneously

hypertension rat contributes to increased oxidative stress. *Hypertension* 45: 786–792, 2005
32. Salzler HR, Griffiths R, Ruiz P, Chi L, Frey C, Marchuk DA, et al.: Hypertension and albuminuria in chronic kidney disease mapped to a mouse chromosome 11 locus. *Kidney Int* 72: 1226–1232, 2007
33. Cechova S, Zeng Q, Billaud M, Mutchler S, Rudy CK, Straub AC, et al.: Loss of collectrin, an angiotensin-converting enzyme 2 homolog, uncouples endothelial nitric oxide synthase and causes hypertension and vascular dysfunction. *Circulation* 128: 1770–1780, 2013
34. Gigliotti JC, Huang L, Ye H, Bajwa A, Chattrabhuti K, Lee S, et al.: Ultrasound prevents renal ischemia-reperfusion injury by stimulating the splenic cholinergic anti-inflammatory pathway. *J Am Soc Nephrol* 24: 1451–1460, 2013
35. Cechova S, Dong F, Chan F, Kelley MJ, Ruiz P, Le TH: *MYH9* E1841K mutation augments proteinuria and podocyte injury and migration. *J Am Soc Nephrol* 29: 155–167, 2018
36. Fawzi WW, Rifas-Shiman SL, Rich-Edwards JW, Willett WC, Gillman MW: Calibration of a semi-quantitative food frequency questionnaire in early pregnancy. *Ann Epidemiol* 14: 754–762, 2004
37. Tang L, Paonessa JD, Zhang Y, Ambrosone CB, McCann SE: Total isothiocyanate yield from raw cruciferous vegetables commonly consumed in the United States. *J Funct Foods* 5: 1996–2001, 2013
38. Rebholz CM, Coresh J, Ballew SH, McMahon B, Whelton SP, Selvin E, et al.: Kidney failure and ESRD in the Atherosclerosis Risk in Communities (ARIC) study: Comparing ascertainment of treated and untreated kidney failure in a cohort study. *Am J Kidney Dis* 66: 231–239, 2015
39. Schlöndorff D, Banas B: The mesangial cell revisited: No cell is an island. *J Am Soc Nephrol* 20: 1179–1187, 2009
40. Kriz W, Shirato I, Nagata M, LeHir M, Lemley KV: The podocyte's response to stress: The enigma of foot process effacement. *Am J Physiol Renal Physiol* 304: F333–F347, 2013
41. Schordan S, Schordan E, Endlich K, Endlich N: AlphaV-integrins mediate the mechanoprotective action of osteopontin in podocytes. *Am J Physiol Renal Physiol* 300: F119–F132, 2011
42. Taniyama Y, Griendling KK: Reactive oxygen species in the vasculature: Molecular and cellular mechanisms. *Hypertension* 42: 1075–1081, 2003
43. Just A, Olson AJ, Whitten CL, Arendshorst WJ: Superoxide mediates acute renal vasoconstriction produced by angiotensin II and catecholamines by a mechanism independent of nitric oxide. *Am J Physiol Heart Circ Physiol* 292: H83–H92, 2007
44. Trott DW, Harrison DG: The immune system in hypertension. *Adv Physiol Educ* 38: 20–24, 2014
45. Olson E, Pravenec M, Landa V, Koh-Tan HHC, Dominiczak AF, McBride MW, et al.: Transgenic overexpression of glutathione S-transferase μ-type 1 reduces hypertension and oxidative stress in the stroke-prone spontaneously hypertensive rat. *J Hypertens* 37: 985–996, 2019
46. Whaley-Connell AT, Sowers JR, Stevens LA, McFarlane SI, Shlipak MG, Norris KC, et al.; Kidney Early Evaluation Program Investigators: CKD in the United States: Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES) 1999-2004. *Am J Kidney Dis* 51[Suppl 2]: S13–S20, 2008
47. Bernardini S, Hirvonen A, Järventaus H, Norppa H: Trans-stilbene oxide-induced sister chromatid exchange in cultured human lymphocytes: Influence of GSTM1 and GSTT1 genotypes. *Mutagenesis* 16: 277–281, 2001
48. Berhane K, Widersten M, Engström A, Kozarich JW, Mannervik B: Detoxication of base propenals and other alpha, beta-unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases. *Proc Natl Acad Sci U S A* 91: 1480–1484, 1994
49. Hubatsch I, Ridderström M, Mannervik B: Human glutathione transferase A4-4: An alpha class enzyme with high catalytic efficiency in the conjugation of 4-hydroxynonenal and other genotoxic products of lipid peroxidation. *Biochem J* 330: 175–179, 1998
50. Paumi CM, Smitherman PK, Townsend AJ, Morrow CS: Glutathione S-transferases (GSTs) inhibit transcriptional activation by the peroxisomal proliferator-activated receptor gamma (PPAR gamma) ligand, 15-deoxy-delta 12,14prostaglandin J2 (15-d-PGJ2). *Biochemistry* 43: 2345–2352, 2004
51. Kistler AD, Altintas MM, Reiser J: Podocyte GTPases regulate kidney filter dynamics. *Kidney Int* 81: 1053–1055, 2012
52. Alexis NE, Zhou H, Lay JC, Harris B, Hernandez ML, Lu TS, et al. The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. *J Allergy Clin Immunol* 124: 1222–1228.e5, 2009
53. Miyake M, Goodison S, Urquidi V, Gomes Giacoia E, Rosser CJ: Expression of CXCL1 in human endothelial cells induces angiogenesis through the CXCR2 receptor and the ERK1/2 and EGF pathways. *Lab Invest* 93: 768–778, 2013
54. Hung CC, Chang CT, Chen KH, Tian YC, Wu MS, Pan MJ, et al.: Upregulation of chemokine CXCL1/KC by leptospiral membrane lipoprotein preparation in renal tubule epithelial cells. *Kidney Int* 69: 1814–1822, 2006
55. Bodonyi-Kovacs G, Ma JZ, Chang J, Lipkowitz MS, Kopp JB, Winkler CA, et al.: Combined effects of GSTM1 null allele and APOL1 renal risk alleles in CKD progression in the African American Study of Kidney Disease and Hypertension trial. *J Am Soc Nephrol* 27: 3140–3152, 2016
56. Reeves-Daniel AM, DePalma JA, Bleyer AJ, Rocco MV, Murea M, Adams PL, et al.: The APOL1 gene and allograft survival after kidney transplantation. *Am J Transplant* 11: 1025–1030, 2011

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