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Evidence for a Causal Role of the *SH2B3*-β_2M Axis in Blood Pressure Regulation: the Framingham Heart Study

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Abstract

Genetic variants at *SH2B3* are associated with blood pressure and circulating beta-2 microglobulin (β_2M), a well-characterized kidney filtration biomarker. We hypothesize that circulating β_2M is an independent risk predictor of hypertension and may causally contribute to its development.

The study sample consisted of 7065 Framingham Heart Study participants with measurements of plasma β_2M. Generalized estimating equations were used to test the association of β_2M with prevalent and new-onset hypertension. There were 2145 (30%) cases of prevalent hypertension at baseline and 886 (21%) cases of incident hypertension during six years of follow-up. A 1-standard deviation increase in baseline plasma β_2M was associated with a greater risk of prevalent (odds ratio [OR] 1.14, 95% confidence interval [CI] 1.05–1.24) and new-onset (OR 1.18, 95% CI 1.07–1.32) hypertension. Individuals within the top β_2M quartile had a greater risk than the bottom quartile for prevalent (OR 1.29, 95% CI .05–1.57) and new-onset (OR 1.59, 95% CI 1.20–2.11) hypertension. These associations remained essentially unchanged in analyses restricted to participants free of albuminuria and chronic kidney disease (CKD). Mendelian randomization demonstrated that lower *SH2B3* expression is causal for increased circulating β_2M levels, and in a hypertensive mouse model, knockout of *Sh2b3* increased *β_2M* gene expression.

In a community-based study of healthy individuals, higher plasma β_2M levels are associated with increased risk of prevalent and incident hypertension independent of CKD status. Overlapping

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Disclosures

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genetic signals for hypertension and β_2M, in conjunction with mouse knockout experiments, suggest that the *SH2B3*-β_2M axis plays a causal role in hypertension.

Summary

Based on overlapping genetic associations, we explored the association between β_2M and hypertension, and demonstrate β_2M to be associated with hypertension both cross-sectionally and longitudinally in Framingham Heart Study participants with and without impaired kidney function. Furthermore, using Mendelian randomization and knockout of *Sh2b3* in a mouse model of hypertension, we provide evidence that *trans*-regulation of β_2M by *SH2B3* may play a causal role in blood pressure regulation.

Keywords

Hypertension; β_2M; biomarkers; gene expression; genome-wide association study; epidemiology

Introduction

Beta-2-microglobulin (β_2M), an essential component of the class I major histocompatibility complex (MHC),[1,2] is freely filtered in the glomerulus and reabsorbed in the proximal tubule, with circulating levels tightly maintained in healthy individuals.[3] A prior genome-wide association study (GWAS) of circulating β_2M identified genetic variants at the *MHC* and *SH2B3* genetic loci[4], with one of the top single nucleotide polymorphisms (SNP) for circulating β_2M at the *SH2B3* locus, rs3184504, also being a genome-wide significant SNP for blood pressure (BP)[5]. Therefore, given the concurrence of genetic variants for BP and circulating β_2M, we hypothesized that β_2M may be a causal factor for hypertension.

Hypertension is preventable through lifestyle interventions and drug treatment,[6,7] but it still remains the most widespread non-communicable disease worldwide.[8,9] Previous population studies of β_2M and hypertension are either limited to cross-sectional analyses[10] or did not consider kidney disease status[11]. To address these limitations, we sought to determine whether plasma β_2M was associated with hypertension cross-sectionally and longitudinally and to assess its potential clinical utility as a prognostic indicator of hypertension risk. We also performed secondary analyses excluding individuals with albuminuria and low estimated glomerular filtration rate (eGFR) at baseline because elevated urinary β_2M levels have been reported to be associated with impaired kidney function[10]. Lastly, we provide results of a functional study in *Sh2b3* knockout mice to explore a causal role of the *SH2B3*-β_2M axis in hypertension.

Materials and Methods

All source data and materials have been made publicly available at dbGaP (accession number phs000363.v17.p11).

Study population:

The cross-sectional study sample consisted of Framingham Heart Study (FHS) Offspring and Third Generation cohort participants.[12,13,14] A total of 7242 participants had measurements of plasma β_2M[15] at the baseline examination (Offspring cohort examination 7 [1999–2003, n=3267] and Third Generation cohort examination 1 [2002–2005, n=3975]). Participants with the following conditions were excluded: myocardial infarction or heart failure prior to baseline; missing information regarding use of anti-hypertensive treatment, lipid lowering treatment, and diabetes medication use; and missing information on cigarette smoking, body-mass index, fasting glucose, total- or high-density lipoprotein cholesterol, triglycerides, or serum creatinine – leaving a final cross-sectional sample size of 7065.

Participants who attended the follow-up examination (Offspring cohort examination 8 [2005–2008] and Third Generation cohort examination 2 [2008–2011]) approximately six years after baseline (median 6.0, 25th and 75th percentiles 6.0, 7.0 years after baseline) and were normotensive at baseline, made up the longitudinal study sample (n=4189). Secondary longitudinal analyses were performed after excluding those with albuminuria (defined as urinary albumin-creatinine ratio [UACR]≥30mg/g) or low eGFR (eGFR<60 mL/min/1.73 m^2) at baseline.

Quantification of plasma β_2M protein:

Plasma β_2M concentration was measured at baseline as part of the Systems Approach to Biomarker Research in Cardiovascular Disease (SABRe CVD) Initiative[16]. A modified sandwich enzyme-linked immunosorbent assay, multiplexed on a Luminex xMAP platform (Sigma-Aldrich)[17] was used, as described previously[18]. All targets were first developed as single assays, after which compatible targets, with appropriate plasma analyte concentration ranges, were combined to form multiplex panels to avoid cross-reactivity. For the β_2M assay, the detection antibody was product #200–406-141s (Rockland Antibodies, Limerick, PA), the capture antibody was GTX20759 (Genetex, Irvine, CA), and the reference protein was #126–11 (Lee BioSolutions, Maryland Heights, MO). The mean intra-assay coefficient of variation for β_2M was 3.7%[17].

Data collection for covariates:

Each FHS examination consisted of an in-person interview, physician-administered physical examination, anthropometric measurements, BP measurements, and blood/urine sample collection. BMI was calculated as weight (kg) divided by the square of height (m^2). Current cigarette smoking was defined as self-reported smoking of at least one cigarette per day, on average, during the year preceding the examination. Information on antihypertensive and lipid lowering medication-use was collected via a self-administered questionnaire and verified using medications that participants brought to the research clinic visit. Diabetes was defined as fasting blood glucose greater than or equal to 126 mg/dL, or the current use of insulin or hypoglycemic medication. Participant spot urine samples were used to measure urinary albumin and creatinine levels, from which the UACR was calculated. Albuminuria was defined as a UACR equal to or greater than 30 mg/g[19]. Serum creatinine levels, measured using the Roche Hitachi 911 reader, were quantified using the modified Jaffe method[20] with a modified picric acid buffer solution to minimize interference and weighted

by population-specific distributions of age and sex[21]. eGFR was calculated using the CKD-epi equation.[22, 23] CKD was defined as eGFR < 60mL/min/1.73m^2.[24]

BP measurement:

BP was measured at the baseline and follow-up examinations. Systolic and diastolic BP were each measured twice by a physician in the left arm after five minutes of rest in the seated position, and mean values were used. Hypertension was defined as systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg, or current antihypertensive medication use[25].

Statistical Analyses:

Generalized estimating equations (GEE) were used to account for familial correlations in testing the association of baseline β_2M concentration with prevalent and new-onset hypertension. In the GEE models, nuclear family members were considered as clusters of measurements. The logit link function was applied for the dichotomous response variable, hypertension. All analyses for prevalent hypertension were adjusted for the following baseline covariates: age, sex, study cohort, BMI, diabetes, cigarette smoking, levels of total and HDL-cholesterol, statin-use, and eGFR. Analyses for new-onset hypertension were adjusted for the same baseline covariates, along with baseline systolic and diastolic BP. Secondary analyses were performed after excluding individuals with prevalent CKD or albuminuria.

Reclassification analyses were conducted by comparing the predictive abilities of regression models with and without plasma β_2M for new-onset hypertension, and used the integrated discrimination improvement (IDI) and net reclassification improvement (NRI) metrics.[26,27] Bootstrapping with 1000 iterations was used for probability estimates,[26] and two-sided *P* values of less than 0.05 were considered statistically significant. All analyses were conducted using SAS software version 9.4.

Mendelian Randomization (MR):

A two-sample inverse-variance weighted MR approach was used to test whether *SH2B3* expression was causal for plasma β_2M levels. Pruned *cis*-eQTL variants (LD r^2<0.1) for *SH2B3* expression[28] were used as instrumental variables (IVs) for *SH2B3* expression with *SH2B3* expression as the exposure and circulating β_2M levels as the outcome. *SH2B3* expression was measured as previously described[28]. Briefly, fasting whole blood samples were collected from FHS participants in PAXgene™ tubes (PreAnalytiX, Hombrechtikon, Switzerland) and stored at −80 °C. Total RNA was isolated from frozen PAXgene tubes by Asuragen, Inc, and used to generate cDNA. The cDNA was fragmented and labeled using the FL-Ovation™ cDNA Biotin Module, after which the resultant cDNA fragments were allowed to hybridize overnight to the Affymetrix human exon 1.0 ST microarray platform according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). The GeneChip™ 7G GCS3000 scanner (Thermo Fisher Scientific, Waltham, MA) was used to laser scan the microarray following washing and staining using the GeneChip™ Fluidics Station 450. Intensity values for each gene chip were compiled and normalized using the robust multi-chip average method from Affymetrix Power Tools Software version 1.12.0 (Affymetrix) before and after quality control.

All MR analyses were conducted using MRbase (http://www.mrbase.org/). Causal effect estimates of *SH2B3* expression on plasma β_2M levels are reported as effect per risk allele on inverse rank-normalized protein level. Because we lacked *cis*-pQTL variants for circulating β_2M levels, we were unable to conduct MR analyses to determine if plasma β_2M is causal for hypertension.

Mouse Models:

Wild-type (WT) C57B1/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). *Sh2b3*-deficient C57BL/6J mice were generated by deleting exons 3–8 of *Sh2b3,* as previously described[29,30]. Osmotic mini-pumps (model 2002, Alzet, DURECT Corporation, City, State) were implanted subcutaneously in mice approximately 8–12 weeks of age for angiotensin II (490 ng/kg/min) or vehicle (sodium chloride/acetic acid solution) infusion for 14 days. RNA was extracted from whole blood of WT and $Sh2b3^{-/-}$ mice using a RiboPure™ RNA purification Kit (Cat# AM1928, Life Technologies, Carlsbad, CA), and cDNA was constructed using VANTAGE (Vanderbilt Technologies for Advanced Genomics, Vanderbilt University Medical Center, Nashville, TN). RNA sequencing reads first underwent quality control (QC) using the FASTX-Toolkit package (http://hannonlab.cshl.edu/fastx_toolkit/), after which they were mapped to the mouse reference genome (UCSC mm10) using Tophat v2.0[31]. mRNA levels were estimated and normalized using Cufflinks v2.2 and are reported in expected number of fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM)[32]. Cuffdiff was used to identify differential expression of β_2M between WT and $SH2B3^{-/-}$ mice (http://cole-trapnell-lab.github.io/cufflinks/cuffdiff/).

Results

Baseline characteristics:

Summary demographic and clinical characteristics of the study sample (mean age 49 years, 54% women) are shown in Table 1 according to β_2M quartiles and in Table S1 for the overall study sample.

Cross-sectional analyses:

Baseline β_2M concentration was positively associated with prevalent hypertension after adjusting for clinical covariates with an odds ratio (OR) of 1.14 (95% confidence interval [CI] 1.05–1.24, P=0.003) per standard deviation increase in log-transformed β_2M, and an OR of 1.29 (95% CI 1.05–1.57, P=0.01) for the top versus bottom β_2M quartile (Table 2).

Longitudinal analyses:

A total of 886 (21%) participants who were normotensive at baseline developed new-onset hypertension after a mean follow-up of six years. Results of the GEE model revealed a significant association between baseline β_2M and risk of new-onset hypertension with an OR of 1.18 (95% CI 1.07–1.32, P=0.001) per standard deviation increase in log-transformed β_2M (Table 3). Results of the quartile analysis showed that the top β_2M quartile had a 1.59-fold risk (95% CI 1.20, 2.11, P=0.001) of incident hypertension versus those in the bottom

quartile (Table 3). Excluding individuals with albuminuria and low eGFR at baseline did not materially change the results (Table S2).

Reclassification Analyses:

Reclassification metrics revealed that β_2M improved the prediction of new-onset hypertension over the clinical model, which consisted of the following baseline covariates: age, sex, study cohort, BMI, diabetes, cigarette smoking, levels of total and HDL-cholesterol, statin-use, eGFR, and systolic and diastolic BP. Addition of β_2M to the clinical model improved the discrimination for new onset hypertension in the overall sample (integrated discrimination improvement [IDI] 0.0021, P=0.03) and in those free of baseline albuminuria and CKD (IDI=0.0036, P=0.008; Table 4). The continuous net reclassification index (NRI) was statistically significant in participants free of baseline albuminuria and low eGFR (NRI 0.030, P=0.02) but not in the general sample (P>0.05; Table 4).

Mendelian randomization:

As the top genetic variant (rs3184504) associated with plasma β_2M levels is also associated with *SH2B3* expression[33], we postulated that the *trans*-effects (>1 megabase upstream or downstream of the transcription start site) of the rs3184504 polymorphism on β_2M levels may be mediated through its *cis*-effects (within 1 megabase) on *SH2B3* expression. To test this hypothesis, we conducted two-sample inverse-variance weighted MR using all independent *cis* variants (LD r^2<0.1) for *SH2B3* expression as instrumental variables, *SH2B3* expression as the exposure, and circulating β_2M levels as the outcome. Decreased *SH2B3* expression was found to be causal for higher β_2M levels (β=−16.8, P=8.7E-11; Fig. 1).

Sh2b3$^{-/-}$ mouse model:

To recapitulate the effects of *cis* genetic variation within *SH2B3*, we tested the effects of *SH2B3* deficiency on *β_2M* expression at baseline and in response to angiotensin II-induced hypertension. The rs3184504 polymorphism is associated with reduced *SH2B3* function in humans,[34] and we previously reported that *Sh2b3*$^{-/-}$ mice had a greater hypertensive response and more severe renal/vascular dysfunction in response to angiotensin II infusion[35]. Analysis of RNA sequencing performed on whole blood from wild type and *Sh2b3*$^{-/-}$ mice revealed a significant induction of *β_2M* expression in response to angiotensin II administration only in *Sh2b3*$^{-/-}$ mice (P=0.037; Fig. 2) suggesting that *SH2B3* normally constrains *β_2M* expression in response to hypertensive stimuli and that enhanced *β_2M* expression may be causally related to the exaggerated hypertensive response and end-organ damage seen in *Sh2b3*$^{-/-}$ animals.

Discussion

Motivated by GWAS variants at the *SH2B3* locus that were reported to be associated both with BP[5] and circulating β_2M levels[4], we first explored the association of circulating β_2M with prevalent and incident hypertension in a large community-based cohort study. We found that circulating β_2M was associated with clinically relevant increases in prevalent and new-onset hypertension, replicating association results previously reported by investigators from

the Atherosclerosis Risk in Communities study[11]. Secondary longitudinal association analyses between β_2M and hypertension conducted in FHS participants free of baseline CKD and albuminuria demonstrated similar effect sizes and consistent directions of effect compared to the overall study sample, thus suggesting β_2M is more than a marker of renal dysfunction. Our MR analyses and mechanistic study in mice suggest that the *SH2B3*-β_2M axis may play a causal role in BP regulation. Reclassification analyses, however, reveal that whereas the addition of β_2M to a clinical model improved hypertension prediction, the magnitude of effect suggests that circulating β_2M level is not likely to be a clinically useful biomarker of hypertension risk.

Hypertension is a complex disorder with a prominent renal component characterized by impaired renal sodium excretion, vascular remodeling, inflammation, and fibrosis.[36,37] Genes within the *HLA* and *SH2B3* regions have been implicated in inflammation,[38] and recent *Sh2b3*-knockout studies in mice have linked mutations or deletions in *SH2B3* with kidney inflammation[39], hypertension[30,40,41], and atherosclerotic CVD[34,42,43, 44]. Therefore, we sought to determine if there was a mechanistic link between *SH2B3*, β_2M, and hypertension. To this end, we first conducted MR and found that lower *SH2B3* expression increases circulating β_2M levels (Fig. 1). Due to a lack of *cis* genetic variants for circulating β_2M, we were unable to conduct MR to determine whether circulating β_2M is causal for hypertension. We previously reported that *Sh2b3*$^{-/-}$ mice develop an exaggerated BP response and more severe end-organ damage (renal and vascular inflammation and dysfunction) in response to angiotensin II[30]. We extend these findings in the present study by reporting that β_2M expression is significantly increased in response to angiotensin II infusion in *Sh2b3*$^{-/-}$ but not wild type mice (Fig. 2). We thus provide evidence for a causal role of the *SH2B3*-β_2M axis in hypertension.

To our knowledge, ours is the first study to demonstrate that *SH2B3* might regulate hypertension not only via production of its coded protein, the lymphocyte adaptor protein (LNK), but also through effects on β_2M expression. Deficiency of LNK increases interferon gamma producing CD8$^+$ T-cells in the spleen and kidneys of hypertensive mice, and both interferon gamma and CD8+ T cells are mediators of hypertension[30,45]. β_2M is essential for MHC-I-mediated CD8$^+$ T-cell activation[46]. Our finding that loss of LNK increases β_2M expression under hypertensive conditions is consistent with our MR results. We hypothesize that loss of *SH2B3* and a resultant increase in β_2M levels likely leads to increased CD8+ T cell activation and interferon gamma production, which promote hypertension and hypertensive end-organ damage (Fig. 1).

Our study has several strengths. First, the sample size was large and adequate to assess the relations of B2M to prevalent and incident hypertension. Second, BP and eGFR were meticulously ascertained, minimizing recall bias. Third, association results for prevalent and incident hypertension largely replicate those of an independent external study[11].

We acknowledge several study limitations. First, circulating β_2M was only measured at the baseline examination, preventing analyses of temporal variation in plasma β_2M. In addition, this was a European ancestry study sample; the extent to which our results are indicative of what would be observed in other racial/ethnic groups is unknown. Third, a substantial

proportion of participants did not have measurement of urinary albumin, resulting in a smaller sample size for secondary longitudinal analyses that excluded those with CKD or albuminuria. Fourth, we applied an inclusive definition for hypertension that included elevated BP or antihypertensive medication use. Finally, we were unable to conduct MR of plasma β_2M in relation to hypertension due to a lack of *cis* genetic variants for circulating β_2M.

In summary, we have identified β_2M to be cross-sectionally and longitudinally associated with hypertension risk independent of albuminuria and kidney disease and provide functional evidence of a causal role of the *SH2B3*-β_2M axis in hypertension. Future studies are needed to further understand the mechanistic role of β_2M in hypertension and determine if there is therapeutic efficacy in targeting the *SH2B3*-β_2M axis for hypertension treatment or prevention.

Perspectives

β_2M is cross-sectionally and longitudinally associated with hypertension in Framingham Heart Study participants with and without kidney dysfunction. Mendelian randomization analysis and studies using a murine model of hypertension provide evidence for a causal role of the *SH2B3*-β_2M axis in blood pressure regulation. Future studies are needed to determine whether β_2M is a clinically-useful prognostic indicator of hypertension and whether there is therapeutic utility in targeting the *SH2B3*-β_2M axis for hypertension prevention and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What is New?

To our knowledge, no study to-date has analyzed in detail the role of the *SH2B3*-β_2M axis in blood pressure regulation. Moreover, population studies of associations between β_2M and hypertension are either limited to cross-sectional analyses or do not consider kidney disease status.

What is Relevant?

We provide novel evidence of a causal role of the *SH2B3*-β_2M axis in blood pressure regulation, which can be further explored as a potential therapeutic target in hypertension.

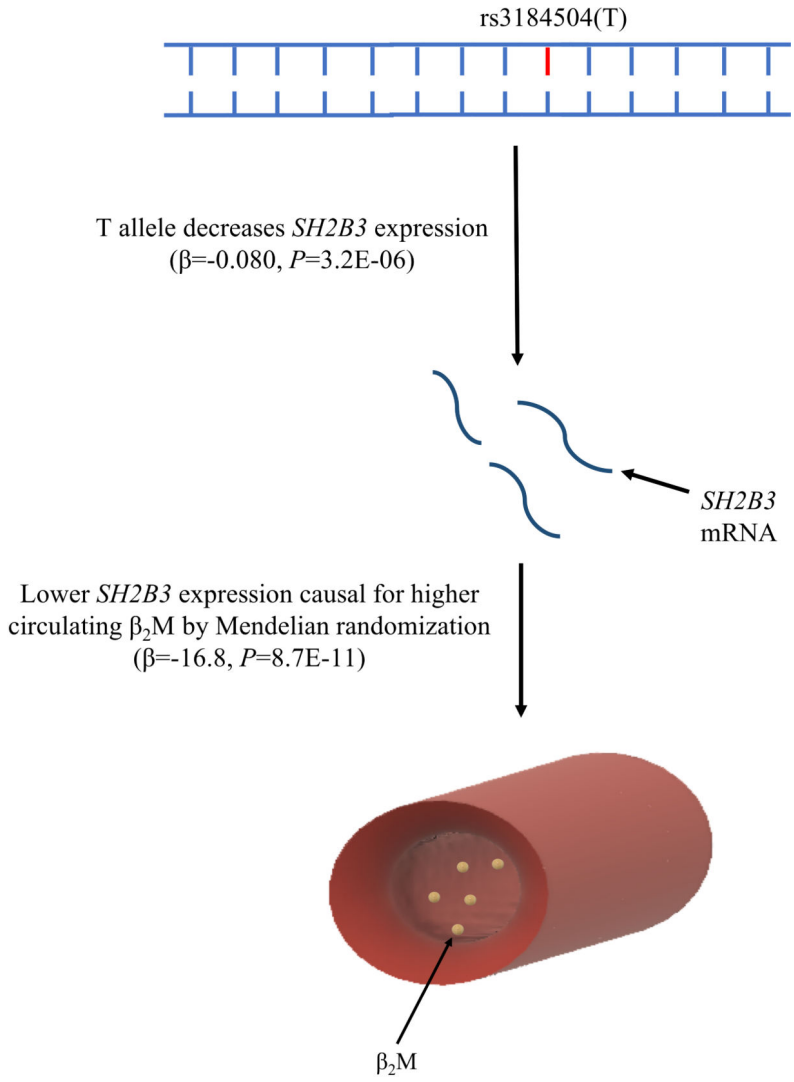

Figure 1. Mendelian randomization demonstrating *SH2B3* expression to be causal for plasma β_2M levels and how both increase hypertension risk

Mendelian randomization (MR) analyses were conducted using rs3184504 to instrument *SH2B3* expression to determine the causal effect of *SH2B3* expression on circulating β_2M levels. rs3184504(T) is a *cis*-eQTL variant for *SH2B3* expression (β=−0.08, *P*=3.2E-06), *trans*-pQTL variant for circulating β_2M (β=0.12, *P*=9.7E-11), and GWAS SNP associated with systolic (β=0.58, *P*=5E-09) and diastolic (β=0.48, *P*=3E-14) blood pressure[35]. The MR Wald ratio demonstrated that *SH2B3* expression was causal for circulating β_2M (β=−16.8, *P*=8.7E-11).

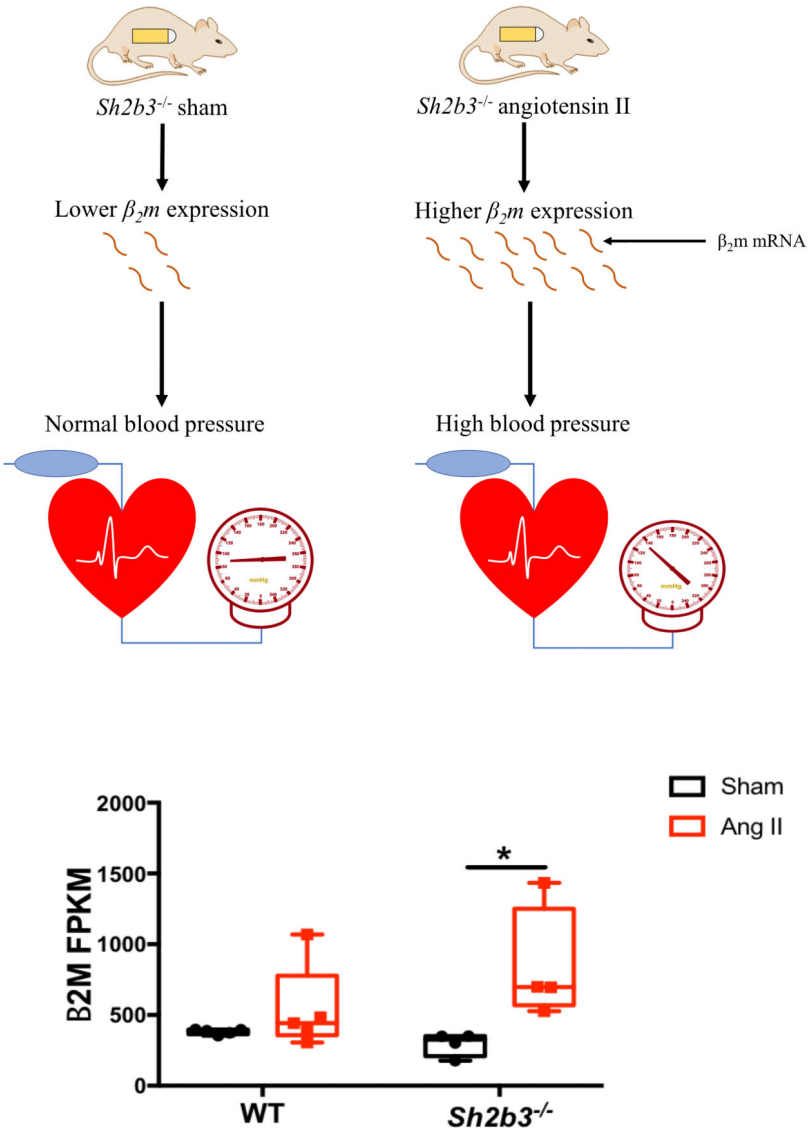

Figure 2. Angiotensin II infusion significantly increases β_2M expression in *Sh2b3*$^{-/-}$ mice

WT and *Sh2b3*$^{-/-}$ mice[47] were infused with vehicle (sham) or angiotensin II (Ang II) for 2 weeks. We previously showed that *Sh2b3*$^{-/-}$ mice develop exaggerated hypertension in response to Ang II infusion. RNA sequencing analysis demonstrated that whole blood β_2M transcripts are increased in *Sh2b3*$^{-/-}$ mice, but not WT mice, in response to Ang II-induced hypertension (P=0.037 by 2-way ANOVA).

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Table 1.

Demographic and clinical characteristics of all FHS study participants by β_2M quartile (N=7065)

β_2M Quartile	**1**		**2**		**3**		**4**		**Trend (*P*)**
N	1794		1751		1754		1766		
β_2M (min,max, mg/L)	1.03	1.19	1.3	1.4	1.49	1.62	1.81	2.2	
	Mean or number	SD or percent	Mean or number	SD or percent	Mean or number	SD or percent	Mean or number	SD or percent	
Age (year)	42.3	10.4	46.1	12.0	50.8	13.0	58.7	13.8	**0.0066**
BMI (kg/m^2)	26.0	4.8	27.2	5.2	27.9	5.5	28.8	6.0	**0.0002**
Systolic BP (mmHg)	116.5	14.8	119.3	15.5	122.5	16.9	127.1	19.3	0.73
Diastolic BP (mmHg)	74.3	9.8	75.4	9.3	75.6	9.6	73.9	10.0	**0.0001**
Total cholesterol (mg/dL)	190.7	35.1	195.1	36.0	196.2	35.5	194.0	37.4	**0.0002**
HDL cholesterol (mg/dL)	58.1	16.8	55.0	16.3	52.9	16.0	50.5	16.1	**0.014**
Fasting blood sugar (mg/dL)	94.8	16.9	96.9	18.7	100.2	23.2	104.6	28.3	0.61
eGFR (mL/min/1.73 m^2)	103.5	13.1	98.5	14.4	92.2	15.2	81.1	19.4	0.40
Women (n,%)	1049	58.5	897	51.2	899	51.3	948	53.7	**0.012**
Hypertension treatment (n,%)	165	9.2	265	15.1	381	21.7	661	37.4	**0.005**
Hypertension (n,%)	306	17.1	418	23.9	557	31.8	864	48.9	**0.0014**
Lipid-lowering treatment (n,%)	167	9.3	177	10.1	276	15.7	367	20.8	0.12
Diabetes treatment (n, %)	24	1.3	41	2.3	70	4.0	140	7.9	**0.057**
Diabetes (n,%)	43	2.4	75	4.3	134	7.6	206	11.7	0.25
Cigarette smoker (n,%)	294	16.4	245	14.0	249	14.2	217	12.3	0.45
CKD (n,%)	4	0.22	9	0.51	29	1.7	235	13.3	**<.0001**
Albuminuria (n,%)	51	3.04	48	3.06	66	4.33	156	10.8	**0.0004**
UACR (median, Q1/Q3,mg/g)	4.41	2.76/8.82	4.06	2.56/7.77	4.22	2.64/8.09	5.25	2.92/ 11.96	0.41

Linear *P* trend values are adjusted for baseline age, sex, and study cohort. All *P* values less than 0.05 are shown in **bold**.

Abbreviations: BMI=body mass index; BP=blood pressure; HDL=high density lipoprotein; eGFR=estimated glomerular filtration rate; CKD=chronic kidney disease (defined as eGFR<60 mL/min/1.73m^2); UACR=urinary albumin-creatinine ratio (albuminuria defined as UACR≥30 mg/g)

Table 2.

Odds ratios for prevalent hypertension by β_2M levels

β_2M levels	**Cases/non-cases**	**Odds ratio**	**95% CI**		***P* value**
Continuous Model					
Log β_2M (per 1 SD)	2145/4920	1.14	1.05	1.24	**0.0026**
Quartile Model					
β_2M quartile 1	306/1488	Ref			
β_2M quartile 2	418/1333	1.03	0.86	1.23	0.75
β_2M quartile 3	557/1197	1.04	0.87	1.24	0.67
β_2M quartile 4	864/902	1.29	1.05	1.57	**0.013**

P values less than 0.05 are shown in **bold**.

Abbreviations: CI=confidence interval; SD= standard deviation

Table 3.

Odds ratios for new-onset hypertension by β_2M levels

β_2M levels	**Cases/non-cases**	**Odds ratio**	**95% CI**		***P* value**
Continuous Model					
Log (β_2M(mg/L))	886/3303	1.18	1.07	1.32	**0.0011**
Quartile Model					
β_2M quartile 1	153/1103	Ref			
β_2M quartile 2	194/953	0.95	0.71	1.28	0.76
β_2M quartile 3	268/761	1.3	0.99	1.7	0.057
β_2M quartile 4	271/486	1.59	1.20	2.11	**0.0011**

P values less than 0.05 are shown in **bold**.

Abbreviations: CI=confidence interval; SD= standard deviation

Table 4.

Reclassification analyses for new-onset hypertension

Integrated Discrimination Improvement (IDI)				
Study population (N-cases/N-total)	**IDI**	**SE**	***P* value**	**95% CI**
Total sample (886/4189)	0.0021	0.001	**0.029**	(0.0002,0.0041)
Individuals free of baseline albuminuria and CKD (663/3638)	0.0036	0.0014	**0.0084**	(0.0009,0.0063)
Net Reclassification Index (NRI)				
Study population (N-cases/N-total)	**NRI**	**SE**	***P* value**	**95% CI**
Total sample (886/4189)	0.0073	0.0088	0.41	(−0.01,0.025)
Individuals free of baseline albuminuria and CKD (663/3638)	0.030	0.012	**0.017**	(0.0055,0.0535)

P values less than 0.05 are shown in **bold**.

The clinical model for hypertension consisted of the following baseline covariates: age, sex, study cohort, BMI, diabetes, cigarette smoking, levels of total and HDL-cholesterol, statin-use, eGFR, and systolic and diastolic blood pressure. For the NRI, the case validation, or additional percentage of events correctly reclassified over clinical model, was 1% in the general sample and 3% in those free of baseline albuminuria and CKoD; the non-case validation, or additional percentage of non-events correctly reclassified over the clinical model, was 0% for the general sample and those free of baseline albuminuria and CKD.

Abbreviations: CI=confidence interval; CKD=chronic kidney disease; IDI=integrated discrimination improvement; NRI=net reclassification index; SE=standard error