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Mutation of *SH2B3* (*LNK*), a GWAS candidate for hypertension, attenuates Dahl SS hypertension via inflammatory modulation

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Abstract

Human genome wide association studies (GWAS) have linked *SH2B3* (*LNK*) to hypertension and renal disease, though little experimental investigation has been done to verify a role for *SH2B3* in these pathologies. SH2B3, a member of the SH2B adaptor protein family, is an intracellular adaptor protein that functions as a negative regulator in many signaling pathways, including inflammatory signaling processes. To explore a mechanistic link between *SH2B3* and hypertension, we targeted the *SH2B3* gene for mutation on the Dahl salt-sensitive (SS) rat genetic background with zinc-finger nucleases (ZFN). The resulting mutation was a 6 base-pair, in-frame deletion within a highly-conserved region of the Src Homology 2 (SH2) domain of *SH2B3*. This mutation significantly attenuated Dahl salt-sensitive (SS) hypertension and renal disease. Also, infiltration of leukocytes into the kidneys, a key mediator of Dahl SS pathology, was significantly blunted in the Sh2b3^{em1Mcwi} mutant rats. To determine if this was due to differences in immune signaling, bone marrow transplant studies were performed in which Dahl SS and Sh2b3^{em1Mcwi} mutants underwent total body irradiation and were then transplanted with Dahl SS or Sh2b3^{em1Mcwi} mutant bone marrow. Rats that received Sh2b3^{em1Mcwi} mutant bone marrow had a significant reduction in mean arterial pressure and kidney injury when placed on a high salt diet (4% NaCl). These data further support a role for the immune system as a modulator of disease severity in the pathogenesis of hypertension and provide insight into inflammatory mechanisms at play in human hypertension and renal disease.

Keywords

Hypertension; kidney; immune system; lymphocytes; rat

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DISCLOSURES/CONFLICT OF INTEREST

Sigma and MCW have a license agreement that could send royalties based on rat sales to MCW.

INTRODUCTION

Cardiovascular disease (CVD) was the underlying cause of 1 of every 3 deaths in the US in 2010.[1] CVD also causes major morbidity. Hypertension is a major risk factor for CVD; it affects roughly 1/3 of the adults in the US.[1] The etiology for >90% of cases of hypertension is unknown, contributing to the statistic that only ~50% of hypertensive patients have their blood pressure controlled through current therapies. This observation highlights the importance of continued research on mechanisms involved in blood pressure regulation and the pathophysiology of hypertension in order to develop new therapies for the treatment of this common risk factor for CVD.

Recent genome wide association studies (GWAS) in humans have nominated candidate genes for hypertension, a genetically complex disease trait.[2] The general functions of such candidate genes are often unknown and/or not intuitively linked to hypertension. Exploring the mechanistic relationship between the candidate genes and hypertension may lead to the discovery of novel pathways involved in blood pressure regulation, which may identify therapeutic targets for the treatment of hypertension. The gene *SH2B3 (LNK)* has been linked to human hypertension and renal disease by GWAS.[3,4] This gene encodes SH2B adaptor protein 3 (SH2B3, LNK), an intracellular adaptor protein shown to play a major role in hematopoiesis and cytokine signaling.[5] From N-terminal to C-terminal, SH2B3 contains a proline-rich protein interaction site, a pleckstrin homology (PH) domain, a Src Homology (SH2) domain, and many putative tyrosine phosphorylation sites.[5] Though most functional testing of SH2B3 has been performed in hematopoietic cells and endothelial cells, it is expressed in many other tissues, such as the kidney, brain, and various muscle types. Because of its potential role in inflammatory pathway regulation, we sought to explore the role of *SH2B3* in a well-established model of hypertension, the Dahl salt-sensitive (SS) rat.

Recent experimental and clinical evidence has shown a role for immune-mediated mechanisms in the pathogenesis of hypertension.[6-8] In the Dahl SS rat, the development of hypertension and renal disease is accompanied by a significant accumulation of T lymphocytes and macrophages in the kidneys. Pharmacological or genetic immunosuppression inhibits the infiltration of immune cells in the kidneys and significantly blunts the hypertension and kidney injury.[9-11] Because of *SH2B3*'s expression in immune cells (notably T cells and macrophages) and the importance of inflammatory signaling in the Dahl SS phenotype, we aimed to assess whether the GWAS-nominated gene, *SH2B3*, may be linked to hypertension via inflammatory mechanisms. Targeting of *SH2B3* for mutation on the Dahl SS genetic background allowed us to assess its importance in Dahl SS pathology. Zinc-finger nuclease-mediated genetic mutation led to a 6-bp deletion in a highly-conserved region of *SH2B3*. This deletion is predicted to affect a phosphotyrosine-binding site in the SH2 domain, a region that facilitates signal transduction via interactions with phosphorylated tyrosine residues on other proteins.[12] Because there is significant attenuation of hypertension and kidney injury in the Sh2b3^{em1Mcwi} mutant rats, experiments were performed to confirm that alterations in immune cells directly play a role in the blunted phenotype.

METHODS

Animals

All animal breeding and experimental procedures were performed at the Medical College of Wisconsin following protocols approved by the Institutional Animal Care and Use Committee. The methods regarding the generation of the Sh2b3^{em1Mcwi} mutant rat, phenotyping, and statistical analyses are provided in the online-only Data Supplement.

RESULTS

The 6-bp deletion present in the mutant SH2B3 mRNA does not affect gene expression, but is predicted to alter a highly conserved protein sequence

To test the contribution of *SH2B3* to salt-sensitive hypertension, ZFNs were designed to target a highly conserved sequence within the *SH2B3* gene. We validated the mutation in RNA by designing PCR primers to amplify the mutation site of *SH2B3* in cDNA. Following PCR amplification, the resulting amplicons were separated on a polyacrylamide gel. Ethidium bromide detection revealed the Sh2b3^{em1Mcwi} mutant transcript to be smaller than the Dahl SS transcript, indicating a deletion within the sequence (Figure 1A). The PCR products were then cloned and sequenced, confirming a 6-bp deletion at the mutation site (Figure 1C). This mutation did not alter the mRNA expression of SH2B3 in the kidney or peripheral blood mononuclear cells compared to the Dahl SS (Figure 1B); however, the mutation was predicted to modify protein sequence. As shown in Figure 1D, the mutated amino acids (red) are evolutionarily conserved among all 83 analyzed vertebrate species (bottom) and are located in the phospho-peptide binding site in the SH2 domain (top, JAK2 shown as a representative phosphotyrosine binding partner). The native proline-leucine-glutamic acid sequence is replaced with a single glutamine in the Sh2b3^{em1Mcwi} mutant rat, resulting in modification of the phosphotyrosine-peptide binding pocket.

Mutation of SH2B3 expands immune cell compartments and increases the percentage of T regulatory cells (Tregs) within the T cell population

Because of SH2B3's importance in immune cell development, differentiation, and signaling, changes in global immune system characteristics were determined in Sh2b3^{em1Mcwi} mutant rats maintained on 0.4% NaCl or fed 4% NaCl for 3 weeks (n=4-7/grp). Compared to the Dahl SS rats, the Sh2b3^{em1Mcwi} mutants had significantly greater spleen weights (2.4 ± 0.2 vs 1.5 ± 0.1g) and showed a significant increase in the number of total peripheral blood mononuclear cells (PBMCs)(14.7± 0.6 vs 5.9 ± 0.9 10^6/ml), including T cells (7.0± 0.4 vs 3.3 ± 0.5 10^6/ml), and B cells (4.0± 0.3 vs 1.0 ± 0.2 10^6/ml) (Figure 2, A). High salt diet had no effect on these values. Independent of the increase in the total number of immune cells, mutation of *SH2B3* significantly increased the percentage of T regulatory cells (CD4+CD25+FOXP3+) within the T cell population in both the circulation (5.2 ± 0.3 vs 3.5 ± 0.1%) and spleen (4.9 ± 0.1 vs 4.0 ± 0.1%)(Figure 2, C & D) compared to the Dahl SS controls. Additionally, high salt diet caused a significant increase in the percentage of Tregs within splenic T cells in the Sh2b3^{em1Mcwi} mutant (5.9 ± 0.2 vs 4.9 ± 0.1%), but not the Dahl SS.

Sh2b3^{em1Mcwi} mutant rats have attenuated hypertension and albuminuria

Perturbations to the immune system have been shown to affect blood pressure and renal function in Dahl SS rats.[9-11] These characteristics were assessed in the Sh2b3^{em1Mcwi} mutant rat to determine if mutation of *SH2B3* affected the progression of Dahl SS pathology. Figure 3 shows the differences in blood pressure and albumin excretion rate between age-matched Dahl SS and Sh2b3^{em1Mcwi} mutants (n=6-8/group) maintained on 0.4% NaCl chow or fed 4.0% NaCl chow for 21 days. There was no difference in mean arterial pressure (MAP) between Dahl SS and Sh2b3^{em1Mcwi} mutants maintained on 0.4% NaCl chow (127.7 ± 2.0 vs. 127.5 ± 1.5 mmHg); but, the Sh2b3^{em1Mcwi} mutants fed 4.0% NaCl chow for 21 days had significantly lower blood pressure than did Dahl SS rats on the same diet (135.7 ± 1.1 vs 175.5 ± 7.9 mmHg). The albumin excretion rate was significantly lower in the Sh2b3^{em1Mcwi} mutants compared to the Dahl SS rats at days 0 (3.6 ± 0.3 vs. 20.0 ± 2.1 mg/day), 10 (9.4 ± 2.4 vs. 101.1 ± 15.4 mg/day), and 21 (30.3 ± 7.0 vs. 104.9 ± 8.9 mg/day) of 4.0% NaCl diet. These data indicate that mutation of *SH2B3* significantly attenuates Dahl SS hypertension and renal disease.

Sh2b3^{em1Mcwi}mutant rats have reduced renal damage and blunted infiltration of immune cells in the kidney

Figure S1 illustrates the renal histological changes due to increased salt intake of the Dahl SS and Sh2b3^{em1Mcwi} mutant rats (n=5-6/group). As expected, the Dahl SS rats developed substantial renal injury in response to high salt diet, including significant glomerular damage and outer medullary damage (blocked and dilated tubules). These features were not present in the Sh2b3^{em1Mcwi} mutants fed high salt. In confirmation of our previous reports,[9,13,14] Dahl SS rats fed 4.0% NaCl chow for 21 days had a significantly greater infiltration of leukocytes (CD45+ cells) into the kidneys (3.92 ± 0.42 vs. 2.13 ± 0.36 10^6/kidney), including T cells (CD3+ cells)(0.42 ± 0.04 vs 0.21 ± 0.03 10^6/kidney), compared to Dahl SS rats maintained on 0.4% NaCl (Figure 4). In contrast to the observations in the Dahl SS, no differences in immune cell infiltration in the kidney were observed between Sh2b3^{em1Mcwi} mutants fed 0.4% NaCl and those fed 4.0% NaCl chow. Furthermore, Sh2b3^{em1Mcwi} mutant rats on high salt had significantly less renal infiltration of immune cells than Dahl SS rats on high salt (1.84 ± 0.30 vs 3.92 ± 0.42 10^6/kidney).

Mutation of SH2B3 does not affect vascular reactivity

As mentioned above, SH2B3 is expressed in endothelial cells. In order to determine whether preserved vascular function contributes to the attenuation of hypertension in the Sh2b3^{em1Mcwi} mutant rats, we measured vascular reactivity in resistance vessels isolated from rats fed 0.4% NaCl or 4.0% NaCl diet. We postulate that any changes in vascular function due to the mutation of SH2B3 would be ubiquitous. Therefore we utilized mesenteric arteries as proxies to resistance vessels throughout the body. Figure S2 illustrates vascular reactivity of small mesenteric arteries as measured by wire myography (n=4-7/group). Regardless of rat genotype (Dahl SS or Sh2b3^{em1Mcwi}) or diet (0.4% NaCl or 4.0% NaCl), there were no significant differences among the groups in vasoconstriction to serotonin (5-HT) or vasodilation to the endothelial-dependent dilator acetylcholine (ACh).

Mutation of SH2B3 attenuates hypertension and albuminuria via the hematopoietic compartment

Due to the reduced accumulation of immune cells in the kidneys and the blunted pathology in Sh2b3^{em1Mcwi} mutant rats, experiments were performed to determine if discrepancies in immune cell function between Dahl SS and Sh2b3^{em1Mcwi} mutants could account for the attenuated phenotype. The role of immune cells in the disease phenotype could be tested by transferring immune cell compartments between the genotypes via bone marrow transplants. Following total body irradiation (TBI), Dahl SS and Sh2b3^{em1Mcwi} mutant rats (n=5-6/group) underwent bone marrow transplantation, receiving either Dahl SS or Sh2b3^{em1Mcwi} mutant bone marrow. Figure 5 shows that regardless of the recipient's genotype, those rats that received the Sh2b3^{em1Mcwi} mutant bone marrow had a significant attenuation of hypertension during the last week of the high salt treatment compared to the rats that received the Dahl SS bone marrow. Likewise, once placed on high salt chow, significant elevation of MAP compared to low salt values was delayed in rats with Sh2b3^{em1Mcwi} mutant bone marrow compared to rats with Dahl SS bone marrow. Rats with Sh2b3^{em1Mcwi} mutant bone marrow also had attenuated albuminuria compared to the Dahl SS counterparts on days 14 and 21 of high salt chow. Collectively, these data indicate that the Sh2b3^{em1Mcwi} mutant bone marrow cells are contributing to the attenuation of hypertension and renal damage.

The majority of PBMCs in the transplanted rats are of donor genotype

Following 21 days of high salt treatment, the BMT groups were euthanized for tissue analysis. Having measured significant differences in the phenotypes among the BMT groups, experiments were performed to determine the effectiveness of the BMTs in each group. To do so, RNA was extracted from PBMCs and converted to cDNA. The cDNA samples were analyzed via real-time PCR with genotype-specific primers. The primers were designed in order to determine the presence of Dahl SS and Sh2b3^{em1Mcwi} mutant genotypes in the circulating immune compartment, as shown in figure S3. The 3' end of the Dahl SS forward primer contains the 6 bases that are not present in Sh2b3^{em1Mcwi} mutant transcript. Likewise, the Sh2b3^{em1Mcwi} mutant forward primer excludes the 6 deleted bases in its sequence. The genotype-specific forward primers share a reverse primer. By normalizing the genotype-specific Ct values to Ct values obtained from an SH2B3 primer set flanking the mutation (amplifies both forms of SH2B3 mRNA), the percentage composition of Dahl SS PBMCs and Sh2b3^{em1Mcwi} mutant PBMCs could be determined in the transplanted rats (n=4-6)(Figure S3, bottom). The slight detection of the Sh2b3^{em1Mcwi} mutant transcript in the Dahl SS control is due to non-specific amplification at high Ct values and has very little effect on the overall trend of chimeric bone marrow reconstitution.

DISCUSSION

SH2B3 was shown to be associated with hypertension and renal disease in human GWAS via a SNP in the coding region of the pleckstrin homology domain.[3,4] The aim of our studies was not to recapitulate the GWAS SNP, but to explore the role of *SH2B3* in hypertension and renal disease. Therefore the coding region of the SH2 domain was ultimately targeted for mutation on the Dahl SS genetic background because: 1) this region was determined to

be the optimal site for successful mutation using zinc-finger nuclease technology and 2) the SH2 domain is well characterized for binding partners and function[5] – information that could guide future molecular studies in this model. The 6-bp deletion in the Sh2b3^{em1Mcwi} mutant rat resulted in a significant attenuation of Dahl SS pathology, showing a direct role for *SH2B3* in hypertension and kidney injury. Although protein modeling predicts altered functionality of the SH2 domain in the mutant rats, the exact molecular changes to protein function (loss of function, gain of function, etc.) are unclear.

Testing of vascular reactivity revealed no differences in dilatory or contractile ability between the two strains, suggesting that alterations in intrinsic vascular function do not contribute to the attenuated hypertension in the Sh2b3^{em1Mcwi} mutant rats, though release of local vasoactive agents (possibly by immune cells) during the progression of disease could potentiate hypertension. Furthermore, Dahl SS or Sh2b3^{em1Mcwi} mutants that underwent TBI and were repopulated with Sh2b3^{em1Mcwi} mutant bone marrow had significantly lower blood pressure and albuminuria than the rats repopulated with Dahl SS bone marrow. These studies indicate an attenuation of the Dahl SS pathology via alterations to the immune cell compartment, though the exact mechanism(s) remain elusive.

SH2B3 is a negative regulator of lymphopoiesis, as indicated by the enhanced production of lymphocytes in Lnk-/- mice[15] and reduced lymphopoiesis in mice overexpressing SH2B3 (LNK).[16] We show that mutation of *SH2B3* augments lymphocyte development, yet attenuates hypertension. This may seem counterintuitive when considering the abundance of data that indicate a pathogenic role for the immune system in hypertension. We have shown, through numerous experimental approaches, that immunosuppression attenuates Dahl SS hypertension.[9-11] Many other labs have reported the same phenomenon in their respective models of hypertension as well.[6] Therefore, one might expect that expansion of the immune system would exacerbate hypertension. Interestingly, the kidney seems to be the main site of deleterious inflammation during the pathogenesis of Dahl SS hypertension, incurring significant infiltration of macrophages and T lymphocytes in the renal interstitium.[9,14] The Sh2b3^{em1Mcwi} mutant rats on a high salt diet have blunted renal infiltration of immune cells and attenuated renal disease and hypertension. The trafficking of immune cells from the bloodstream into tissue involves cell-cell interactions with the endothelium.[17] SH2B3 has been shown to modulate hematopoietic cell adhesion to vascular adhesion molecule 1.[18] Mutation of *SH2B3* may inhibit pathways that regulate the trafficking of immune cells to target organs such as the kidney, thus protecting them from deleterious inflammation.

Another plausible mechanism of blunted pathology in the Sh2b3^{em1Mcwi} mutant rats involves T regulatory cells (Tregs). Tregs are a subtype of CD4+ T cells that suppress innate and adaptive immune signaling.[19] Currently the most discriminatory marker for Tregs is the transcription factor forkhead box P3 (FOXP3), an essential component of Treg differentiation in the thymus.[20] Another characteristic often associated with Tregs is the constitutive expression of interleukin 2 receptor α-subunit (CD25) on the cell surface, though it is postulated that mature CD4+FOXP3+CD25-cells are dormant Tregs that upregulate CD25 expression upon homeostatic expansion and/or activation.[21] We detect both CD25+ and CD25- Tregs in the Dahl SS and Sh2b3^{em1Mcwi} mutant rats. The balance

between Tregs and pro-inflammatory T cell subsets is important in maintaining immune homeostasis and a deficiency of Tregs leads to autoimmune disease.[22]

Limited experimental studies suggest a therapeutic role for Tregs in hypertension. Increasing the global expression of Tregs among immune cells via adoptive transfer of exogenous Tregs blunted vascular inflammation and attenuated Ang II-induced hypertension and vascular disease in mice.[23] Apart from the increase in total T cells, mutation of *SH2B3* selectively increases the percentage of Tregs within the splenic and circulating T cell populations. Moreover high salt diet further increases this selective expression of splenic Tregs in the Sh2b3^{em1Mcwi} mutant rats. Considering immune homeostasis as a balance between pro- and anti-inflammatory signaling, the increased ratio of Tregs to pro-inflammatory T cells may provide additional protection from deleterious chronic inflammation. The ratio of Tregs to pro-inflammatory T cells in the circulation has been shown to decrease in patients with rheumatoid arthritis,[24] a condition known to be mediated by the immune system. Furthermore, this ratio has been shown to decrease in diseases of supposed inflammatory mediation, such as minimal change nephrotic syndrome[25] and acute coronary syndrome.[26] In the case of hypertension, increased Treg/pro-inflammatory T cell ratios, as seen in the Sh2b3^{em1Mcwi} mutant rats, may suppress tissue inflammation and attenuate disease.

Though T cells play a major role in the development of Dahl SS hypertension, the exact mechanisms responsible for hypertension and organ damage are unknown. By no means should the influence of other inflammatory cell types be disregarded when considering the development of hypertensive pathology. It is known that there is much crosstalk among cells capable of inflammatory signaling. SH2B3 functions in many signaling pathways that are responsible for a multitude of inflammatory activity.[5] Although the mutation in *SH2B3* resulted in a reduction of Dahl SS pathology via immune cells, the mechanism(s) responsible remain elusive. Furthermore, the expression pattern and function of SH2B3 in many tissues and cell types remains unclear. Although we detected *SH2B3* mRNA in renal cortical and outer medullary tissue, the effects of its mutation on renal physiology are unknown, much less the innate role of SH2B3 in kidney function. It is possible that mutation of *SH2B3* alters blood pressure regulation and renal function via non-immune mechanisms in the mutant rat. Further studies will need to determine the signaling pathways affected by the mutated SH2 domain of SH2B3 to elucidate downstream targets that result in the attenuation of hypertension and kidney injury. Because of the strong association between *SH2B3* and human hypertension and renal disease, the Sh2b3^{em1Mcwi} mutant rat will allow for further dissection of inflammatory mechanisms involved in these pathologies.

PERSPECTIVES

These studies provide experimental validation of the association between *SH2B3* and human hypertension and renal disease described in GWAS. A role for inflammation in the pathogenesis of hypertension is becoming widely accepted, as much data from a variety of experimental models supports this hypothesis.[6-8] These studies indicate that inflammatory pathways could become therapeutic targets for the treatment of human hypertension.

Elucidating molecular mechanisms by which *SH2B3* modulates Dahl SS hypertension will give insight into possible mechanisms at play in the human condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NOVELTY AND SIGNIFICANCE

What is new?

Mutation of *SH2B3* significantly attenuated Dahl SS hypertension via immune cell function, which provides experimental validation of the association between *SH2B3* and hypertension proposed in human GWAS.

What is relevant?

Treatment of hypertension is often not optimal. The studies in this manuscript, along with many others, highlight the immune system as a possible therapeutic target, though much work still remains to elucidate inflammatory pathways that mediate hypertension.

Summary

SH2B3, a GWAS candidate for hypertension and renal disease, encodes an intracellular adaptor protein that functions in many signaling cascades. With no understanding of its association to hypertension, we sought to explore its role in the Dahl SS pathology. Mutation of the highly conserved SH2 domain in the SH2B3 protein resulted in a significant attenuation of hypertension and renal disease. Further experimentation indicated a direct role for immune cells in the altered pathology, though the mechanisms remain unclear.

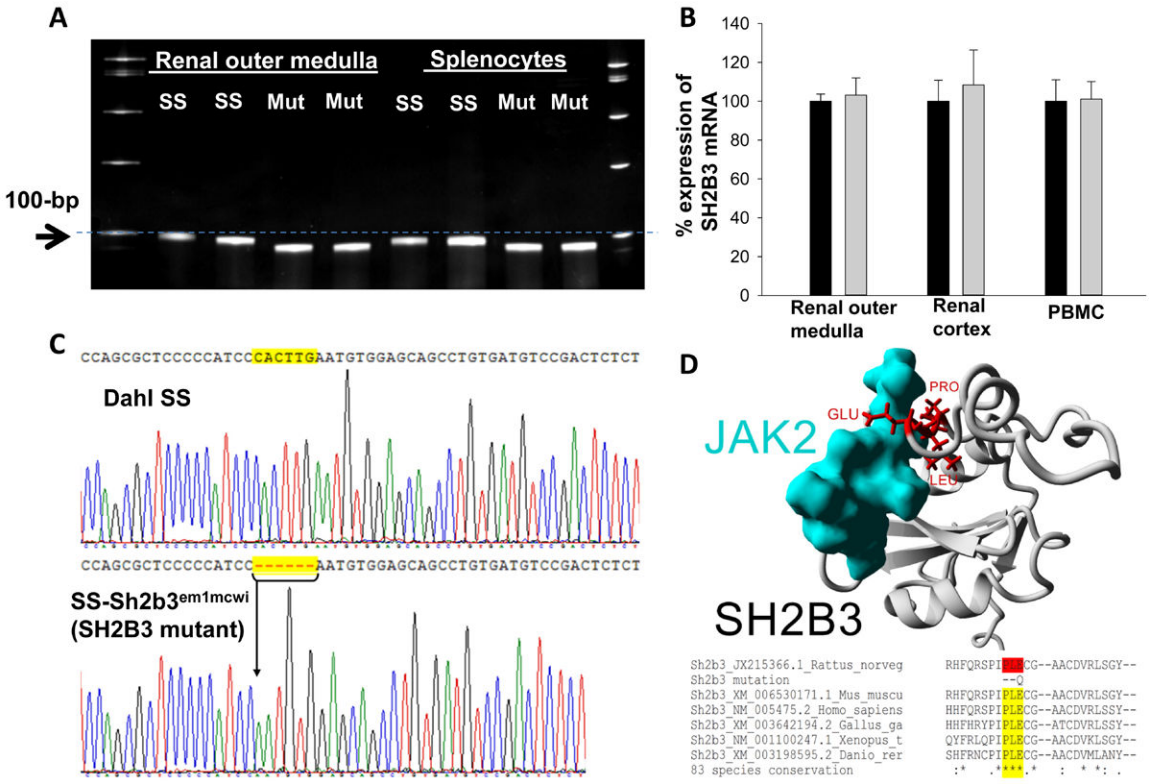

Figure 1.

Detection of cDNA amplicons from primers flanking the mutation site reveal a shift in size between the Dahl SS and Sh2b3^{em1Mcwi} mutant rats, indicating a deletion within the *SH2B3* transcript (A). Real-time PCR shows no difference in *SH2B3* expression between the Dahl SS and Sh2b3^{em1Mcwi} mutant rats in the kidney or PBMCs (B, n=3-6/grp). Sequencing of the cDNA PCR products reveals a 6-bp deletion at the mutation site in the Sh2b3^{em1Mcwi} mutant rats (C). The predicted mutated amino acids (D, top, listed as Glu, Pro, and Leu) are highly conserved among vertebrates (D, bottom) and are located in the phospho-peptide binding site of the SH2 domain (D, top) of SH2B3. A representative phospho-JAK2 is shown in this binding site.

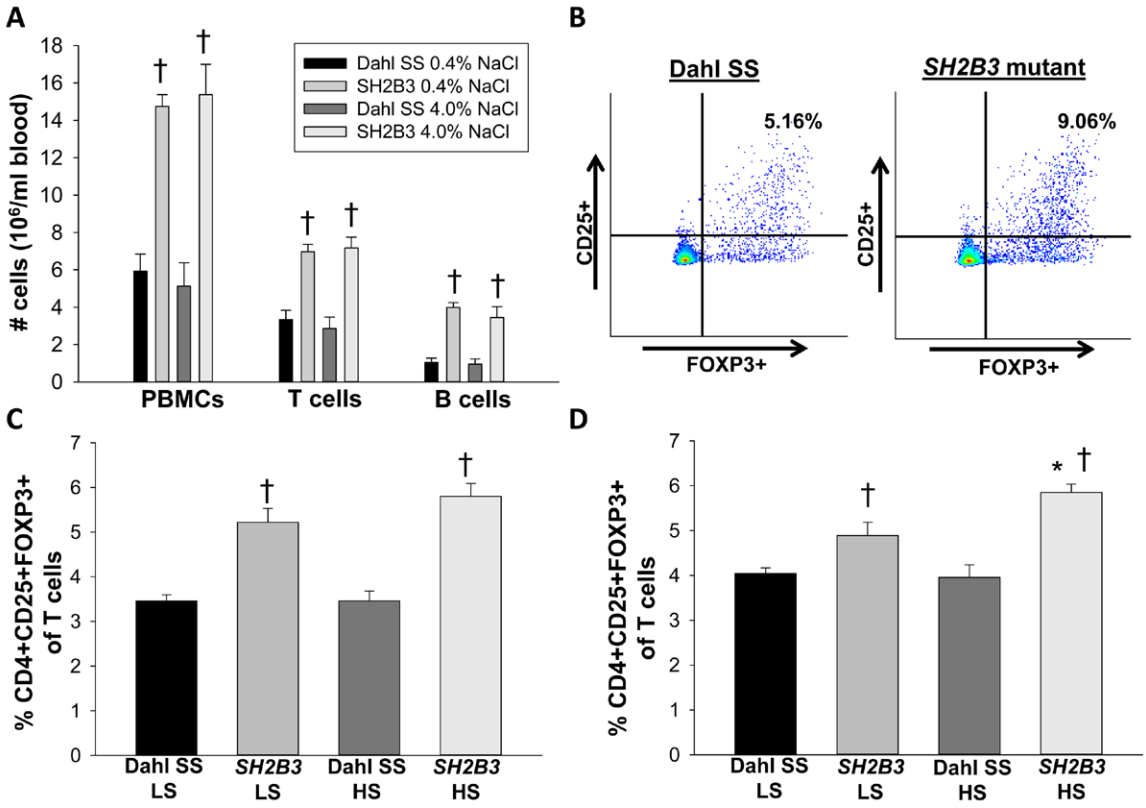

Figure 2.

Quantification of peripheral blood mononuclear cells (A) of age-matched Dahl SS and Sh2b3^{em1Mcwi} mutant rats. Representative scatter plots from flow cytometric analysis displaying CD3+CD4+ PBMCs from Dahl SS and Sh2b3^{em1Mcwi} mutant rats (B). Percent expression of circulating (C) and splenic (D) Tregs (CD4+CD25+FOXP3+) within the T cell population of Dahl SS and Sh2b3^{em1Mcwi} mutant rats maintained on 0.4% NaCl (LS) or 4.0% NaCl (HS) chow. * indicates $p<0.05$ vs. values obtained on 0.4% NaCl chow, † indicates $p<0.05$ vs. Dahl SS on the same diet.

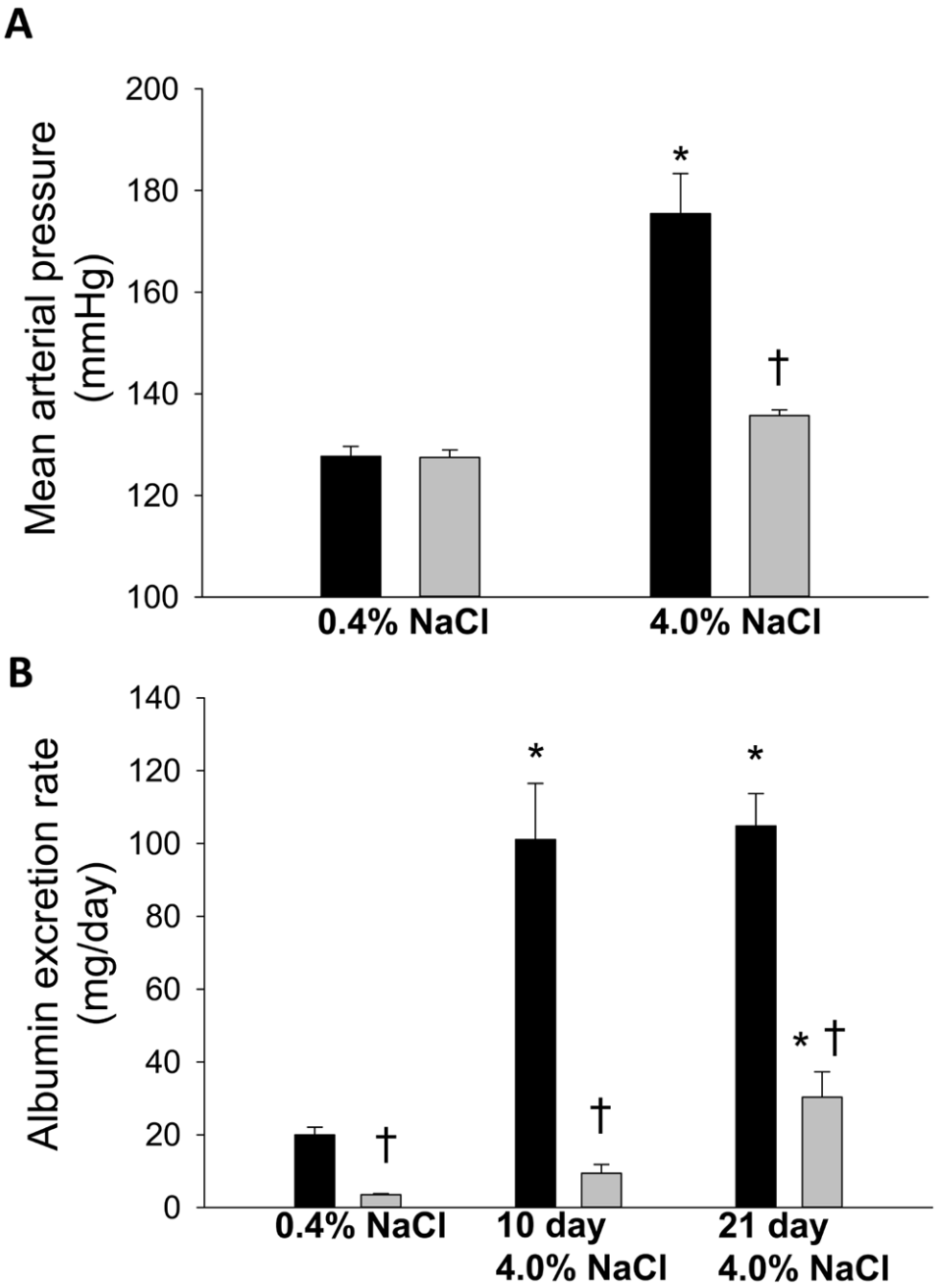

Figure 3.

Mean arterial pressure (A) and albumin excretion rate (B) of age-matched Dahl SS and Sh2b3^{em1Mcwi} mutant rats maintained on 0.4% NaCl chow or fed 4.0% NaCl chow for 21 days. * indicates $p<0.05$ vs. values obtained on 0.4% NaCl chow, † indicates $p<0.05$ vs. Dahl SS on the same diet.

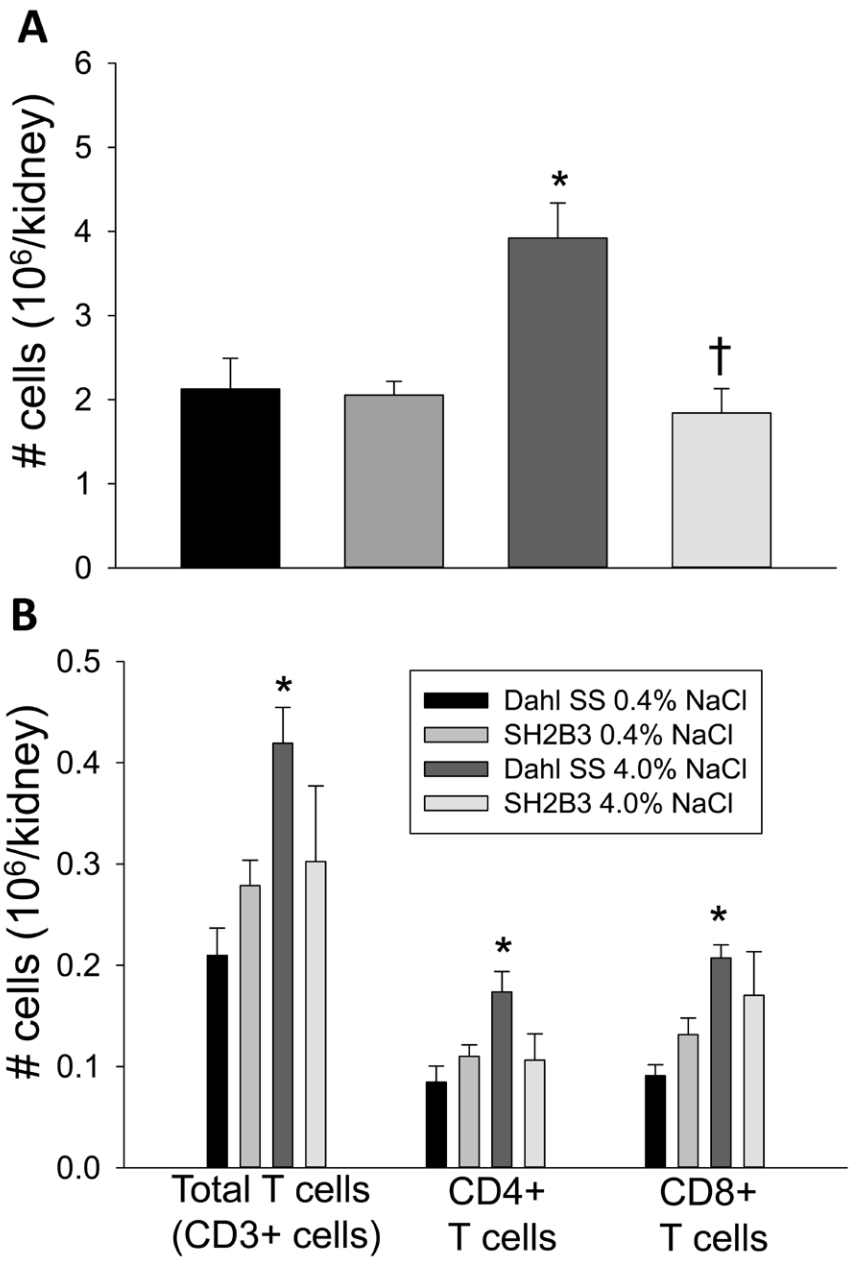

FIGURE 4.

Quantification of total leukocytes (CD45+ cells)(A) and T lymphocytes (CD3+ cells)(B) in the kidneys of age-matched Dahl SS and Sh2b3^{em1Mcwi} mutant rats maintained on 0.4% NaCl chow or fed 4.0% NaCl chow for 21 days. * indicates $p<0.05$ vs. values obtained on 0.4% NaCl chow, † indicates $p<0.05$ vs. Dahl SS on the same diet.

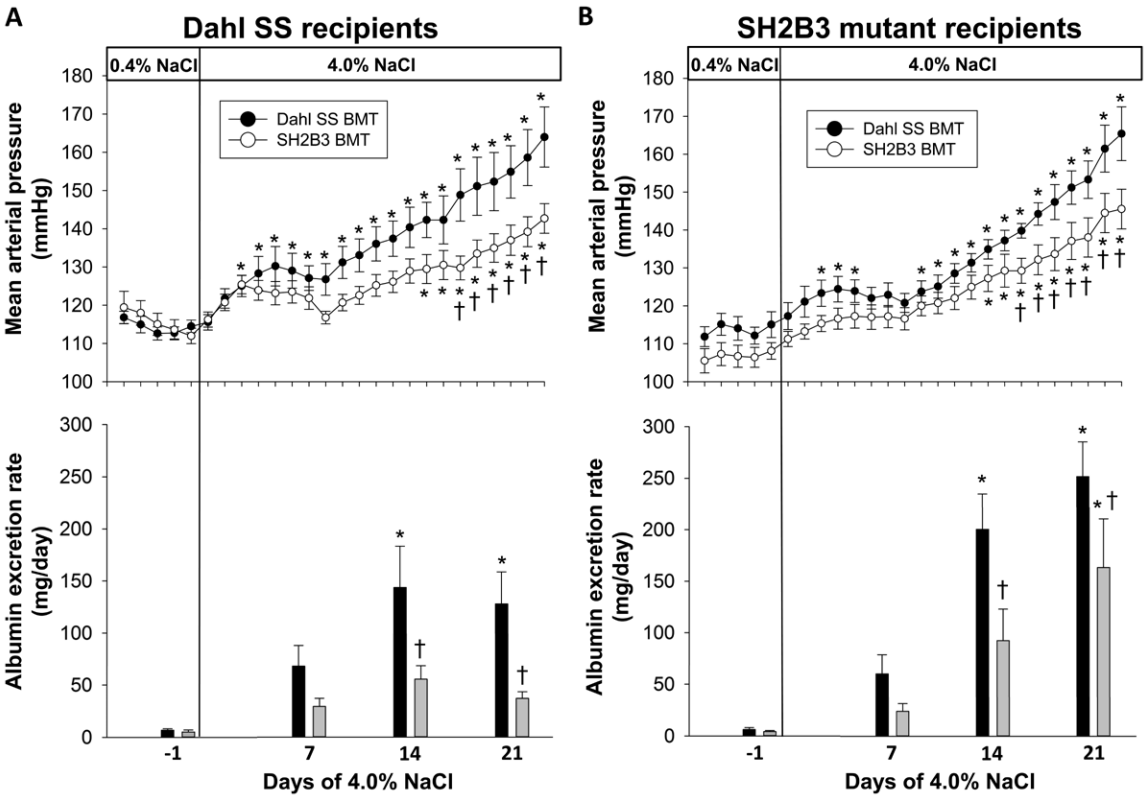

FIGURE 5.

Mean arterial pressure (top) and albumin excretion rate (bottom) in Sh2b3^{em1Mcwi} mutant rats (A) and Dahl SS rats (B) having undergone bone marrow transplants. Rats received either Dahl SS or Sh2b3^{em1Mcwi} mutant bone marrow and were fed 0.4% NaCl chow followed by 21 days of 4.0% NaCl chow. * indicates $p<0.05$ vs. values obtained on the last day of 0.4% NaCl chow, † indicates $p<0.05$ vs. Dahl SS on the same diet.