Published in final edited form as:

Transl Res. 2015 April ; 165(4): 505–511. doi:10.1016/j.trsl.2014.07.006.

The renal dopaminergic system: novel diagnostic and therapeutic approaches in hypertension and kidney disease

Ines Armando¹, Prasad Konkalmatt¹, Robin A. Felder², and Pedro A. Jose^{1,3}

¹Division of Nephrology, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201

²Department of Pathology, The University of Virginia School of Medicine, Charlottesville, VA 22908

³Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

Abstract

Salt sensitivity of blood pressure, whether in hypertensive or normotensive subjects, is associated with increased cardiovascular risk and overall mortality. Salt sensitivity can be treated by reducing NaCl consumption. However, decreasing salt intake in some may actually increase cardiovascular risk, including an increase in blood pressure, i.e., inverse salt sensitivity. Several genes have been associated with salt sensitivity and inverse salt sensitivity. Some of these genes encode proteins expressed in the kidney that are needed to excrete a sodium load, e.g., dopamine receptors and their regulators, GRK4. We review here research in this field that has provided several translational opportunities, ranging from diagnostic tests to gene therapy, such as: 1) a test in renal proximal tubule cells isolated from the urine of humans that may determine the salt-sensitive phenotype by analyzing the recruitment of dopamine D₁ receptors to the plasma membrane; 2) presence of common GRK4 gene variants that are not only associated with hypertension but may also be predictive of the response to antihypertensive therapy; 3) genetic testing for polymorphisms of the dopamine D₂ receptor that may be associated with hypertension and inverse salt sensitivity and may increase the susceptibility to chronic kidney disease because of loss of the anti-oxidant and anti-inflammatory effects of the renal dopamine D₂ receptor, and 4) *in vivo* renal selective amelioration of renal tubular genetic defects by a gene transfer approach, using AAV vectors introduced to the kidney by retrograde ureteral infusion.

© 2014 Mosby, Inc. All rights reserved.

Corresponding author: Pedro A. Jose, MD, PhD, University of Maryland School of Medicine, Department of Medicine, Division of Nephrology, 20 Penn Street- HSFII Suite S003D, Baltimore, MD 21201, Phone: 410-706-6015, pjose@medicine.umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

All authors have read the journal's policy on disclosure of potential conflicts of interest and declare no conflicts of interest

All authors have read the journal's authorship agreement and have reviewed and approved the manuscript.

Introduction

Hypertension is a leading cause of morbidity and mortality and disproportionately affects disadvantaged populations. The prevalence of hypertension is about 40% in low- to upper-middle-income countries and 35% in high income countries (1). In US adults, 20 years of age, hypertension is present in 28.5% in those without cardiovascular disease and 51% in those with cardiovascular disease (2). Salt (NaCl) intake is one of the important determinants of blood pressure and salt sensitivity of blood pressure represents a major hypertension phenotype, as approximately one-half of subjects with essential hypertension are salt-sensitive (3). About one-quarter of normotensive individuals is also salt-sensitive. Salt sensitivity is an insidious “silent killer” since even in individuals with normal blood pressure it can lead to cardiovascular morbidity and mortality (3) and is associated with other diseases, e.g., asthma (4), gastric carcinoma (5), osteoporosis (6), and renal dysfunction (7), among others. High sodium intake can aggravate the morning surge of blood pressure even in salt-resistant subjects (8) that could be associated with increased cardiovascular risk, in some studies (9). Although some studies suggest that non-dipping hypertension associated with low morning surge could also be associated with decreased cardiovascular risk (10), high sodium intake is also associated with non-dipping hypertension which is associated with increased cardiovascular risk (11).

Dietary guidelines on sodium intake

Government and non-profit health-based entities have generally recommended a significant reduction in NaCl consumption (12, 13). The adequate and upper limit of NaCl intake per day as defined by the Institute of Medicine is 3.7–5.8 G (1.5–2.3 G sodium) for young adults and 2.08 – <5.8 for older and elderly (≥ 60 years old) adults (12). Lowering sodium intake can decrease blood pressure and lower cardiovascular risk (14, 15, 16) and progression of kidney disease (17, 18, 19, 20). However, in 2013, a committee of the Institute of Medicine concluded that there was no “evidence for benefit and some evidence suggesting risk of adverse health outcomes associated with sodium intake levels in ranges approximately 1,500 to 2,300 mg/day among those with diabetes, kidney disease, or CVD” (cardiovascular disease). Furthermore, the evidence on both the benefit and harm is not strong enough to indicate that these subgroups should be treated differently than the general U.S. population. “Thus, the evidence on direct health outcomes does not support the recommendations to lower sodium intake within these subgroups to or even below 1,500 mg/day.” (21) The Trials of Hypertension Prevention Study confirmed the health benefits of reducing sodium intake to the 1500 to 2300 mg/day range in the majority of the population, although the data in the lower age range were sparse (22). Indeed, there is no lower limit of NaCl intake; dietary sodium restriction may not be beneficial to everyone as shown by a recent meta-analysis, that did not include the latest data from the Trials of Hypertension Prevention Study, re-affirming the existence of a J- or U-shaped curve with increased morbidity and mortality above and below the adequate NaCl intake advocated by the Institute of Medicine (20). In other words, there is a subset of the population in which a low sodium diet puts the individual at risk for increased cardiovascular disease. Another confounder is the intake of other nutrients, e.g., increased intake of both sodium and potassium may not be associated with higher blood pressures (23) since potassium is thought to be protective. Although the

concern regarding the ability of a low sodium diet to alter lipid metabolism has not been borne out (24), salt depletion may promote insulin resistance and activation of the renin-angiotensin and the sympathetic nervous systems (25, 26), especially those with diabetes (27–29) or heart failure (21, 28, 29). Salt restriction has also been reported to decrease cognitive function in salt-sensitive rats (30). Not usually included in these reports is that in some humans, a low salt intake can increase in blood pressure. This, we have termed inverse salt sensitivity (31–33) which may be found in 11% of the population (33). Polymorphisms of the dopamine D₂ receptor may be associated with inverse salt sensitivity (34) (*vide infra*).

Role of G protein-coupled receptor kinase 4 and dopamine receptors in the regulation of blood pressure and response to antihypertensive treatment

It is difficult to predict whether or not a particular dietary modification or medication is beneficial for blood pressure control to a specific individual. However, pharmacogenomics (drug-treatment based on ones genes), is becoming useful in predicting the beneficial or deleterious outcomes in the treatment of several diseases, including cardiovascular disease (35–38). We and others have reported that G protein-coupled receptor kinase 4 (GRK4) gene variants are involved in the regulation of blood pressure, and may be predictive of response to antihypertensive therapy (39–43). GRK4 is controls blood pressure, in part, by its involvement in the regulation of renal dopamine receptors that are important in the regulation of renal function and blood pressure (38, 44–48).

Dopamine receptors and hypertension

The long-term regulation of blood pressure rests on renal and non-renal mechanisms (49–52). The impaired renal sodium handling in essential hypertension and salt sensitivity could be caused by aberrant counter-regulatory natriuretic and antinatriuretic pathways (49–54). The sympathetic nervous (55, 56) and the renin-angiotensin (50–53) systems are examples of antinatriuretic pathways. An important counter-regulatory natriuretic pathway may be afforded by the renal autocrine/paracrine dopamine system, aberrations of which are involved in the pathogenesis of hypertension (44–49), including that associated with obesity (57–61).

Dopamine, produced locally, in renal tubules, independent of innervation and not metabolized to norepinephrine, decreases renal tubular ion transport in almost all nephron segments (44–49, 62–69). Renal dopamine receptors are responsible for over 50% of the increase in sodium excretion when NaCl intake is increased (70–73). The paracrine/autocrine dopaminergic regulation of sodium excretion is mediated by tubular, rather than by hemodynamic mechanisms (71, 73). Therefore, systemically administered dopaminergic drugs may not mimic the renal autocrine/paracrine function of dopamine.

The importance of renal endogenous dopamine in body sodium homeostasis is demonstrated in mice with genetically altered renal dopamine production. Salt-sensitive hypertension results when renal proximal tubule dopamine production is decreased by the selective deletion in the mouse renal proximal tubule of aromatic amino acid decarboxylase, the enzyme responsible for the production of dopamine in the kidney (63). Dopamine exerts its

actions on the plasma membrane, via two receptor sub-families that belong to the rhodopsin-like family (Class A) of G protein-coupled receptors. D₁-like receptors (D₁R and D₅R in humans, D_{1A} and D_{1B} in rodents) couple to G proteins, G_αS and G_αL, and stimulate adenylyl cyclases. The D₂-like receptors (D₂R, D₃R, and D₄R) couple to inhibitory G proteins, G_αi, and G_αo, inhibit adenylyl cyclases and calcium channels, and modulate potassium channels (39, 44–48, 74). Long-term pharmacological blockade of dopamine receptors decreases renal sodium excretion and increases blood pressure (75–77). Moreover, inactivation of any dopamine receptor subtype gene, in mice, results in hypertension that may be salt-sensitive, depending on the genetic background (39, 44, 45, 49, 74).

Oxidative stress and inflammation and hypertension, role of D₂R

Increased renal reactive oxygen species and renal inflammation have been shown to be important in the pathogenesis of hypertension in several animal models (56, 78–80). Similar to the other dopamine receptor subtypes, the D₂R is also important in the pathogenesis of hypertension (39, 44, 45, 49, 74). Deletion of *Drd2* (D₂^{−/−}) in mice may cause salt-sensitive hypertension that is dependent on genetic background (39, 44, 62, 81, 82, 83, 84, 85, 86). The hypertension in D₂^{−/−} mice is associated with increased renal oxygen species production (62) that may be related to activation of pro-oxidant enzymes, e.g., NADPH oxidase and decreased activation of antioxidant enzymes, including heme oxygenase 2 (62), paraoxonase 2 (83), Parkin or DJ-1 (84) and sestrin 2 (85). The hypertension of D₂^{−/−} mice is also associated with inflammation (39, 86, 87). Silencing *Drd2* in mouse proximal tubule cells increases the expression of pro-inflammatory cytokines and oxidative stress (83, 84, 86).

Human *DRD2* polymorphisms and D₂R expression and function in renal proximal tubule cells

Several common single-nucleotide polymorphisms (SNPs; rs6276 [−141C Ins/Del], rs6277 [957C>T], and rs1800497 [Taq1A polymorphism]), are associated with decreased D₂R expression and function (57, 88–90) (Table 1). *DRD2* rs1800497 is associated with obesity and hypertension (57, 91) and rs6276 (32% minor allele frequency) is associated with salt-sensitive hypertension (34). Human renal proximal tubule cells with *DRD2* SNPs rs6276, rs6277, and rs1800497 have decreased D₂R protein and function and increased expression of inflammatory cytokines and chemokines, e. g., TNF α (Figure 1A), macrophage inflammatory protein-1 β , nuclear factor kappa B1A, interleukin-1F7, interleukin 10, interleukin 22, CCL24, and CXCL14, as well as TGF- β signaling which is important in renal fibrosis associated with epithelial to mesenchymal transition (Figure 1B) and synthesis of extracellular matrix (92). Increasing the D₂R protein expression in these cells with *DRD2* SNPs, by transient transfection of the wild-type *DRD2*, normalized the genes associated with inflammation and renal fibrosis (92).

Renal proximal tubule cells cultured from the urine provide an instantaneous snapshot of *in vivo* phenotype, salt sensitivity

Renal proximal tubule cells are normally shed into the urine (93, 94) and retain their *in vivo* phenotypes (95, 96), including salt sensitivity (31). We and others have reported that a

normal cellular response to D₁R agonist stimulation is the recruitment of D₁R from the cytoplasm to the plasma membrane (44, 97–100). Impairment in D₁R agonist-mediated recruitment of D₁R from the cytoplasm to the plasma membrane in renal proximal tubule cells is associated with defective renal sodium handling in essential hypertension and obesity (44, 97, 98). We have reported that there is also a negative correlation between D₁R recruitment to the plasma membrane of renal proximal tubule cells isolated from the urine of humans with salt sensitivity (31). We have suggested that salt sensitivity, as measured by the above method, is a continuous variable and provide a basis for personalized recommendations of sodium intake based on an individual's unique renal proximal tubule cellular physiology. We also reported that mRNA and miRNA encapsulated in urinary exosomes are differentially expressed in humans with salt sensitivity or inverse salt sensitivity (32). Twenty-four of the 45 miRNAs screened were found to be associated with salt sensitivity or inverse salt sensitivity; miR-4516 was associated with inverse salt sensitivity while miRNA-124 was much higher in salt-sensitive than salt-resistant subjects (32).

***In vivo* amelioration of the high blood pressure of D₂^{-/-} mice**

As aforementioned, D₂^{-/-} mice are hypertensive (81–86) and *DRD2* bearing SNPs in its non-coding region are associated with decreased D₂R expression. The decreased D₂R expression in renal proximal tubule cells from D₂^{-/-} mice or humans with *DRD2* SNPs rs6276, rs6277 and rs1800497 is associated with markers of inflammation and oxidative stress (83, 84, 85, 86). Increasing D₂R expression in these cells ameliorates these abnormal phenotypes, *in vitro* (92). We have preliminary data to suggest that normalizing the renal expression of D₂R can be ameliorated selectively in the kidney *in vivo*. In a preliminary report we found that adeno-associated viral (AAV) vector can provide efficient gene transfer in the kidney with minimum off-target gene expression (Figure 1C) (101). Using this technique we found that the hypertension associated with decreased renal expression of D₂R in mice by the renal subcapsular infusion of *Drd2* siRNA (83–86) was ameliorated by AAV vector carrying wild-type *Drd2* (unpublished). Therefore, the renal tubular selective genetic defect can be ameliorated *in vivo* by a gene transfer approach using AAV vectors and can be made renal tubular segment specific with the use of renal tubular segment specific promoter.

Conclusion

Salt sensitivity of blood pressure, whether or not associated with hypertension, is associated with increased cardiovascular risk and overall mortality. Reduction in NaCl consumption is a treatment of salt sensitivity. However, decreasing salt intake in some may actually increase cardiovascular risk, including an increase in blood pressure. Several genes have been associated with salt sensitivity and inverse salt sensitivity, including those related to genes that express proteins needed to excrete a sodium load, e.g., dopamine receptors and their regulators, GRK4. However, tests to identify the gene that cause salt sensitivity in humans are laborious and expensive. Testing the cellular response to dopamine receptor subtype agonists in renal tubule cells excreted in the urine may be an alternative. One can also determine if an abnormal phenotype can be normalized in these renal tubule cells shed in the

urine. A positive response can then be used as a guide for a renal selective transfer of the normal gene using AAV vectors.

Acknowledgments

The work was funded by grants from the US National Institutes of Health, P01HL074940, P01HL068686, R01HL092196, R37HL023081, R01DK039308, and R01DK090918

Abbreviations

GRK4	G protein-coupled receptor kinase 4
SNP	single nucleotide polymorphism

References

1. Alwan, A., editor. World Health Organization; 2011. Global Status Report on noncommunicable diseases 2010. ISBN 978-92-4-068645-8.
2. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation*. 2014; 129:e28–e292. [PubMed: 24352519]
3. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. *Hypertension*. 2001; 37:429–432. [PubMed: 11230313]
4. Mickleborough TD. Salt intake, asthma, and exercise-induced bronchoconstriction: a review. *Phys Sportsmed*. 2010; 38:118–131. [PubMed: 20424409]
5. Tsugane S, Sasazuki S, Kobayashi M, Sasaki S. Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women. *Br J Cancer*. 2004; 90:128–134. [PubMed: 14710219]
6. Heaney RP. Role of dietary sodium in osteoporosis. *J Am Coll Nutr*. 2006; 25:271S–276S. [PubMed: 16772639]
7. McQuarrie EP, Traynor JP, Taylor AH, et al. Association between urinary sodium, creatinine, albumin, and long-term survival in chronic kidney disease. *Hypertension*. 2014; 64:111–117. [PubMed: 24732890]
8. Osanai T, Okuguchi T, Kamada T, et al. Salt-induced exacerbation of morning surge in blood pressure in patients with essential hypertension. *J Hum Hypertens*. 2000; 14:57–64. [PubMed: 10673733]
9. Li Y, Thijs L, Hansen TW, et al. Prognostic value of the morning blood pressure surge in 5645 subjects from 8 populations. *Hypertension*. 2010; 55:1040–1048. [PubMed: 20212273]
10. Verdecchia P, Angeli F, Mazzotta G, et al. Day-night dip and early-morning surge in blood pressure in hypertension: prognostic implications. *Hypertension*. 2012; 60:34–42. [PubMed: 22585951]
11. Uzu T, Sakaguchi M, Yokomaku Y, et al. Effects of high sodium intake and diuretics on the circadian rhythm of blood pressure in type 2 diabetic patients treated with an angiotensin II receptor blocker. *Clin Exp Nephrol*. 2009; 13:300–306. [PubMed: 19255824]
12. Washington D.C.: Government Publishing Office; 2010. Institute of Medicine, Strategies to Reduce Sodium Intake in the United States, Washington, D.C.: The National Academic Press) (U.S. Department of Agriculture, U.S. Department of Health and Human Services, (2010).
13. Ezzati M, Riboli E. Can noncommunicable diseases be prevented? Lessons from studies of populations and individuals. *Science*. 2012; 337:1482–1487. [PubMed: 22997325]
14. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *BMJ*. 2013; 346:f1325. [PubMed: 23558162]

15. Aburto NJ1, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. *BMJ*. 2013; 346:f1326. [PubMed: 23558163]
16. McMahon EJ1, Bauer JD, Hawley CM, et al. A randomized trial of dietary sodium restriction in CKD. *J Am Soc Nephrol*. 2013; 24:2096–2103. [PubMed: 24204003]
17. He FJ, Pombo-Rodrigues S, Macgregor GA. Salt reduction in England from 2003 to 2011: its relationship to blood pressure, stroke and ischaemic heart disease mortality. *BMJ Open*. 2014; 4:e004549.
18. Aaron KJ, Sanders PW. Role of dietary salt and potassium intake in cardiovascular health and disease: a review of the evidence. *Mayo Clin Proc*. 2013; 88:987–995. [PubMed: 24001491]
19. Frisoli TM, Schmieder RE, Grodzicki T, Messerli FH. Salt and hypertension: is salt dietary reduction worth the effort? *Am J Med*. 2012; 125:433–439. [PubMed: 22482843]
20. Graudal N, Jürgens G, Baslund B, Alderman MH. Compared with usual sodium intake, low- and excessive-sodium diets are associated with increased mortality: a meta-analysis. *Am J Hypertens*. 2014 Apr 1. [Epub ahead of print].
21. Institute of Medicine. Washington, DC: The National Academies Press; 2013. Sodium intake in populations: assessment of evidence.
22. Cook NR, Appel LJ, Whelton PK. Lower levels of sodium intake and reduced cardiovascular risk. *Circulation*. 2014; 129:981–989. [PubMed: 24415713]
23. Rodrigues SL, Baldo MP, Machado RC, Forechi L, Molina M, del C, Mill JG. High potassium intake blunts the effect of elevated sodium intake on blood pressure levels. *J Am Soc Hypertens*. 2014; 8:232–238. [PubMed: 24524886]
24. Harsha DW, Sacks FM, Obarzanek E, et al. Effect of dietary sodium intake on blood lipids: results from the DASH-sodium trial. *Hypertension*. 2004; 43:393–398. [PubMed: 14707154]
25. Brunner HR, Laragh JH, Baer L, et al. Essential hypertension: renin and aldosterone, heart attack and stroke. *N Engl J Med*. 1972; 286:441–449. [PubMed: 4257928]
26. Graudal NA, Galløe AM, Garred P. Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterol. *JAMA*. 1998; 279:1383–1391. [PubMed: 9582047]
27. Anderson CA, Ix JH. Sodium reduction in CKD: suggestively hazardous or intuitively advantageous? *J Am Soc Nephrol*. 2013; 24:1931–1933. [PubMed: 24204000]
28. Bibbins-Domingo K. The institute of medicine report sodium intake in populations: assessment of evidence: summary of primary findings and implications for clinicians. *JAMA Intern Med*. 2014; 174:136–137. [PubMed: 24165962]
29. DiNicolantonio JJ1, Niaz AK, Sadaf R, O' Keefe JH, Lucan SC, Lavie CJ. Dietary sodium restriction: take it with a grain of salt. *Am J Med*. 2013; 126:951–955. [PubMed: 24054177]
30. Ruiz-Opazo N, Lopez LV, Tonkiss J. Modulation of learning and memory in Dahl rats by dietary salt restriction. *Hypertension*. 2004; 43:797–802. [PubMed: 14967836]
31. Gildea JJ, Lahiff DT, Van Sciver RE, et al. A linear relationship between the ex-vivo sodium mediated expression of two sodium regulatory pathways as a surrogate marker of salt sensitivity of blood pressure in exfoliated human renal proximal tubule cells: the virtual renal biopsy. *Clin Chim Acta*. 2013; 421:236–242. [PubMed: 23454474]
32. Gildea JJ, Carlson JM, Schoeffel CD, Carey RM, Felder RA. Urinary exosome miRNome analysis and its applications to salt sensitivity of blood pressure. *Clin Biochem*. 2013; 46:1131–1134. [PubMed: 23726803]
33. Felder RA1, White MJ, Williams SM, Jose PA. Diagnostic tools for hypertension and salt sensitivity testing. *Curr Opin Nephrol Hypertens*. 2013; 22:65–76. [PubMed: 23197156]
34. Carey RM, Schoeffel CD, Gildea JJ, et al. Salt sensitivity of blood pressure is associated with polymorphisms in the sodium-bicarbonate cotransporter. *Hypertension*. 2012; 60:1359–1366. [PubMed: 22987918]
35. Mestroni L, Begay RL, Graw SL, Taylor MR. Pharmacogenetics of heart failure. *Curr Opin Cardiol*. 2014; 29:227–234. [PubMed: 24717669]
36. Johnson JA. Advancing management of hypertension through pharmacogenomics. *Ann Med*. 2012; 44(Suppl 1):S17–S22. [PubMed: 22713143]

37. Munroe PB1, Barnes MR, Caulfield MJ. Advances in blood pressure genomics. *Circ Res.* 2013; 112:1365–1379. [PubMed: 23661711]
38. Thompson MD1, Cole DE, Jose PA. Pharmacogenomics of G protein-coupled receptor signaling: insights from health and disease. *Methods Mol Biol.* 2008; 448:77–107. [PubMed: 18370232]
39. Armando, I.; Jose, PA. Hypertension. In: Murray, MF.; Babyatsky, MW.; Giovanni, MMA., editors. *Clinical Genomics: Practical Applications in Adult Patient Care.* McGraw-Hill Education; 2014. p. 74-80.
40. Bhatnagar V, O'Connor DT, Brophy VH, et al. G-protein-coupled receptor kinase 4 polymorphisms and blood pressure response to metoprolol among African Americans: sex-specificity and interactions. *Am J Hypertens.* 2009; 22:332–338. [PubMed: 19119263]
41. Wagner F, Malice MP, Wiegert E, et al. A comparison of the natriuretic and kaliuretic effects of cicletanine and hydrochlorothiazide in prehypertensive and hypertensive humans. *J Hypertens.* 2012; 30:819–827. [PubMed: 22278145]
42. Rayner B, Ramesar R, Steyn K, Levitt N, Lombard C, Charlton K. G-protein-coupled receptor kinase 4 polymorphisms predict blood pressure response to dietary modification in Black patients with mild-to-moderate hypertension. *J Hum Hypertens.* 2012; 26:334–339. [PubMed: 21544086]
43. Vandell AG, Lobmeyer MT, Gawronski BE, et al. G protein receptor kinase 4 polymorphisms: β -blocker pharmacogenetics and treatment-related outcomes in hypertension. *Hypertension.* 2012; 60:957–964. [PubMed: 22949529]
44. Jose PA, Soares-da-Silva P, Eisner GM, Felder RA. Dopamine and G protein-coupled receptor kinase 4 in the kidney: role in blood pressure regulation. *Biochim Biophys Acta.* 2010; 1802:1259–1267. [PubMed: 20153824]
45. Harris RC, Zhang MZ. Dopamine, the kidney, and hypertension. *Curr Hypertens Rep.* 2012; 14:138–143. [PubMed: 22407378]
46. Asghar M, Tayebati SK, Lokhandwala MF, Hussain T. Potential dopamine-1 receptor stimulation in hypertension management. *Curr Hypertens Rep.* 2011; 13:294–302. [PubMed: 21633929]
47. Aperia A. 2011 Homer Smith Award: To serve and protect: classic and novel roles for Na⁺, K⁺ - adenosine triphosphatase. *J Am Soc Nephrol.* 2012; 23:1283–1290. [PubMed: 22745476]
48. Carey RM. The intrarenal renin-angiotensin and dopaminergic systems: control of renal sodium excretion and blood pressure. *Hypertension.* 2013; 61:673–680. [PubMed: 23407646]
49. Asico L, Zhang X, Jiang J, et al. Lack of renal dopamine D5 receptors promotes hypertension. *J Am Soc Nephrol.* 2011; 22:82–89. [PubMed: 21051739]
50. Gurley SB, Riquier-Brison AD, Schnermann J, et al. AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. *Cell Metab.* 2011; 13:469–475. [PubMed: 21459331]
51. Hall JE, Brands MW, Henegar JR. Angiotensin II and long-term arterial pressure regulation: the overriding dominance of the kidney. *J Am Soc Nephrol.* 1999; 10(Suppl 12):S258–S265. [PubMed: 10201880]
52. Navar LG, Harrison-Bernard LM, Nishiyama A, Kobori H. Regulation of intrarenal angiotensin II in hypertension. *Hypertension.* 2002; 39:316–322. [PubMed: 11882566]
53. Burke M1, Pabbidi MR, Farley J, Roman RJ. Molecular mechanisms of renal blood flow autoregulation. *Curr Vasc Pharmacol.* 2013 Sep 25. [Epub ahead of print].
54. Speed JS, Pollock DM. Endothelin, kidney disease, and hypertension. *Hypertension.* 2013; 61:1142–1145. [PubMed: 23608655]
55. Schlaich MP1, Esler MD, Fink GD, Osborn JW, Euler DE. Targeting the sympathetic nervous system: critical issues in patient selection, efficacy, and safety of renal denervation. *Hypertension.* 2014; 63:426–432. [PubMed: 24324047]
56. Zubcevic J, Jun JY, Kim S, et al. Altered inflammatory response is associated with an impaired autonomic input to the bone marrow in the spontaneously hypertensive rat. *Hypertension.* 2014; 63:542–550. [PubMed: 24366083]
57. Fang YJ, Thomas GN, Xu ZL, Fang JQ, Critchley JA, Tomlinson B. An affected pedigree member analysis of linkage between the dopamine D2 receptor gene TaqI polymorphism and obesity and hypertension. *Int J Cardiol.* 2005; 102:111–116. [PubMed: 15939106]
58. Fu J, Han Y, Wang H, et al. Impaired dopamine D1 receptor-mediated vasorelaxation of mesenteric arteries in obese Zucker rats. *Cardiovasc Diabetol.* 2014; 13:50. [PubMed: 24559270]

59. Alvarez-Aguilar C, Alvarez-Paredes AR, Lindholm B, et al. Effects of dopamine on leptin release and leptin gene (OB) expression in adipocytes from obese and hypertensive patients. *Int J Nephrol Renovasc Dis.* 2013; 6:259–268. [PubMed: 24348062]
60. Kessler RM, Zald DH, Ansari MS, Li R, Cowan RL. Changes in dopamine release and dopamine D2/3 receptor levels with the development of mild obesity. *Synapse.* 2014; 68:317–320. [PubMed: 24573975]
61. Muhammad AB, Lokhandwala MF, Banday AA. Exercise reduces oxidative stress but does not alleviate hyperinsulinemia or renal dopamine D1 receptor dysfunction in obese rats. *Am J Physiol Renal Physiol.* 2011; 300:F98–F104. [PubMed: 20926629]
62. Armando I, Wang X, Villar VA, et al. Reactive oxygen species-dependent hypertension in dopamine D2 receptor-deficient mice. *Hypertension.* 2007; 49:672–678. [PubMed: 17190875]
63. Zhang MZ, Yao B, Wang S, et al. Intrarenal dopamine deficiency leads to hypertension and decreased longevity in mice. *J Clin Invest.* 2011; 121:2845–2854. [PubMed: 21701066]
64. Zaika OL, Mamenko M, Palygin O, Boukelmoune N, Staruschenko A, Pochynyuk O. Direct inhibition of basolateral Kir4.1/5.1 and Kir4.1 channels in the cortical collecting duct by dopamine. *Am J Physiol Renal Physiol.* 2013; 305:F1277–F1287. [PubMed: 23986512]
65. Gildea JJ, Shah IT, Van Sciver RE, et al. The cooperative roles of the dopamine receptors, D₁R and D₅R, on the regulation of renal sodium transport. *Kidney Int.* 2014
66. Grider JS, Ott CE, Jackson BA. Dopamine D1 receptor-dependent inhibition of NaCl transport in the rat thick ascending limb: mechanism of action. *Eur J Pharmacol.* 2003; 473(2–3):185–190. [PubMed: 12892837]
67. Ortiz PA, Garvin JL. Intrarenal transport and vasoactive substances in hypertension. *Hypertension.* 2001; 38:621–624. [PubMed: 11566943]
68. Wang X, Asico LD, Escano C, Armando I, Jones JE, Jose PA. Regulation of renal sodium chloride cotransporter by D3 dopamine receptor. *Kidney Week.* 2012 Abstracts, p82A.
69. Weinman EJ, Biswas R, Steplock D, Douglass TS, Cunningham R, Shenolikar S. Sodium-hydrogen exchanger regulatory factor 1 (NHERF-1) transduces signals that mediate dopamine inhibition of sodium-phosphate co-transport in mouse kidney. *J Biol Chem.* 2010; 285:13454–13460. [PubMed: 20200151]
70. Hansell P, Fasching A. The effect of dopamine receptor blockade on natriuresis is dependent on the degree of hypervolemia. *Kidney Int.* 1991; 39:253–258. [PubMed: 2002639]
71. Siragy HM, Felder RA, Howell NL, Chevalier RL, Peach MJ, Carey RM. Evidence that intrarenal dopamine acts as a paracrine substance at the renal tubule. *Am J Physiol.* 1989; 257:F469–F477. [PubMed: 2528916]
72. Chen CJ, Lokhandwala MF. An impairment of renal tubular DA-1 receptor function as the causative factor for diminished natriuresis to volume expansion in spontaneously hypertensive rats. *Clin Exp Hypertens A.* 1992; 14:615–628. [PubMed: 1352742]
73. Jose PA, Asico LD, Eisner GM, Pocchiari F, Semeraro C, Felder RA. Effects of costimulation of dopamine D1- and D2-like receptors on renal function. *Am J Physiol.* 1998; 275:R986–R994. [PubMed: 9756526]
74. Zeng C, Jose PA. Dopamine receptors: important antihypertensive counterbalance against hypertensive factors. *Hypertension.* 2011; 57:11–17. [PubMed: 21098313]
75. Luippold G1, Zimmermann C, Mai M, et al. Dopamine D₃ receptors and salt-dependent hypertension. *J Am Soc Nephrol.* 2001; 12:2272–2279. [PubMed: 11675403]
76. Haney M, Ward AS, Foltin RW, Fischman MW. Effects of ecopipam, a selective dopamine D1 antagonist, on smoked cocaine self-administration by humans. *Psychopharmacology (Berl).* 2001; 155:330–337. [PubMed: 11441422]
77. Henderson DC, Daley TB, Kunkel L, Rodrigues-Scott M, Koul P, Hayden D. Clozapine and hypertension: a chart review of 82 patients. *J Clin Psychiatry.* 2004; 65:686–689. [PubMed: 15163256]
78. Wilcox CS. Asymmetric dimethylarginine and reactive oxygen species: unwelcome twin visitors to the cardiovascular and kidney disease tables. *Hypertension.* 2012; 59:375–378. [PubMed: 22215715]

79. Montezano AC, Touyz RM. Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies. *Ann Med*. 2012; 44(Suppl 1):S2–S16. [PubMed: 22713144]
80. Harrison DG, Guzik TJ, Lob HE, et al. Inflammation, immunity, and hypertension. *Hypertension*. 2011; 57:132–140. [PubMed: 21149826]
81. Li XX, Bek M, Asico LD, et al. Adrenergic and endothelin B receptor-dependent hypertension in dopamine receptor type-2 knockout mice. *Hypertension*. 2001; 38:303–308. [PubMed: 11566895]
82. Ueda A, Ozono R, Oshima T, et al. Disruption of the type 2 dopamine receptor gene causes a sodium-dependent increase in blood pressure in mice. *Am J Hypertens*. 2003; 16:853–858. [PubMed: 14553965]
83. Yang Y, Zhang Y, Cuevas S, et al. Paraoxonase 2 decreases renal reactive oxygen species production, lowers blood pressure, and mediates dopamine D2 receptor-induced inhibition of NADPH oxidase. *Free Radic Biol Med*. 2012; 53:437–446. [PubMed: 22634053]
84. Cuevas S, Zhang Y, Yang Y, et al. Role of renal DJ-1 in the pathogenesis of hypertension associated with increased reactive oxygen species production. *Hypertension*. 2012; 59:446–452. [PubMed: 22215708]
85. Yang Y, Cuevas S, Yang S, et al. Sestrin2 decreases renal oxidative stress, lowers blood pressure, and mediates dopamine D2 receptor-induced inhibition of ROS production. *Hypertension*. 2014 Jul 14. pii: HYPERTENSIONAHA.114.03840. [Epub ahead of print].
86. Zhang Y, Cuevas S, Asico LD, et al. Deficient dopamine D2 receptor function causes renal inflammation independently of high blood pressure. *PLoS One*. 2012; 7:e38745. [PubMed: 22719934]
87. Shao W, Zhang SZ, Tang M, et al. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via α B-crystallin. *Nature*. 2013; 494:90–94. [PubMed: 23242137]
88. Duan J, Wainwright MS, Comeran JM, et al. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum Mol Genet*. 2003; 12:205–216. [PubMed: 12554675]
89. Thompson J, Thomas N, Singleton A, et al. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics*. 1997; 7:479–484. [PubMed: 9429233]
90. Jönsson EG, Nöthen MM, Grünhage F, et al. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Mol Psychiatry*. 1999; 4:290–296. [PubMed: 10395223]
91. Thomas GN, Tomlinson B, Critchley JA. Modulation of blood pressure and obesity with the dopamine D2 receptor gene TaqI polymorphism. *Hypertension*. 2000; 36:177–182. [PubMed: 10948074]
92. Jiang X, Konkalmatt P, Yang Y, et al. Single-nucleotide polymorphisms of the dopamine D2 receptor increase inflammation and fibrosis in human renal proximal tubule cells. *Hypertension*. 2014; 63:e74–e80. [PubMed: 24379187]
93. Prescott LF. The normal urinary excretion rates of renal tubular cells, leucocytes and red blood cells. *Clin Sci*. 1966; 31:425–435. [PubMed: 5927694]
94. Dörrenhaus A, Müller JI, Golka K, Jedrusik P, Schulze H, Föllmann W. Cultures of exfoliated epithelial cells from different locations of the human urinary tract and the renal tubular system. *Arch Toxicol*. 2000; 74:618–626. [PubMed: 11201669]
95. Racusen LC, Wilson PD, Hartz PA, Fivush BA, Burrow CR. Renal proximal tubular epithelium from patients with nephropathic cystinosis: immortalized cell lines as in vitro model systems. *Kidney Int*. 1995; 48:536–543. [PubMed: 7564123]
96. Rahmoune H, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes*. 2005; 54:3427–3434. [PubMed: 16306358]
97. Sanada H, Jose PA, Hazen-Martin D, et al. Dopamine-1 receptor coupling defect in renal proximal tubule cells in hypertension. *Hypertension*. 1999; 33:1036–1042. [PubMed: 10205244]
98. Trivedi M, Marwaha A, Lokhandwala M. Rosiglitazone restores G-protein coupling, recruitment, and function of renal dopamine D1A receptor in obese Zucker rats. *Hypertension*. 2004; 43:376–382. [PubMed: 14718363]

99. Crambert S, Sjöberg A, Eklöf AC, Ibarra F, Holtbäck U. Prolactin and dopamine 1-like receptor interaction in renal proximal tubular cells. *Am J Physiol Renal Physiol*. 2010; 299:F49–F54. [PubMed: 20462969]
100. Padia SH, Kemp BA, Howell NL, Keller SR, Gildea JJ, Carey RM. Mechanisms of dopamine D₁ and angiotensin type 2 receptor interaction in natriuresis. *Hypertension*. 2012; 59:437–445. [PubMed: 22203736]
101. Konkalmatt PR, Asico LD, Feranil J, Jose PA, Armando I. Efficient in vivo gene transfer to murine renal cells using AAV9 vectors. Abstract submitted. :EB2014.

Figure.

A. Protein expression of TNF α in immortalized human renal proximal tubule cells expressing wild-type *DRD2* (WT), *DRD2* bearing single nucleotide polymorphisms (*DRD2* SNPs) or *DRD2* SNPs transfected with a plasmid encoding wild-type *DRD2* (*DRD2* SNPs + D2R). *P<0.05 vs others, one-way ANOVA, n=4–5/group.

B. Percentage of round- and spindle-shaped human proximal tubule cells bearing either wild-type *DRD2* or *DRD2* SNPs in culture. Spindle-shaped cells are indicative of epithelial to mesenchymal transition.

C. *In vivo* bioluminescence images of a mouse showing AAV-mediated luciferase expression in the kidney. AAV vector carrying fire fly luciferase with CMV promoter was administered by sub-capsular infusion using osmotic mini pump over three days. Seven days post AAV infusion, luciferase expression in the mouse kidney was detected non-invasively using an IVIS-200 imaging system. The relationship between color and image intensity is shown on the right-hand side.

Table 1

Genetic variants of human *DRD2* associated with increased expression of pro-inflammatory and pro-fibrotic proteins in human renal proximal tubule cells.

SNP	rs6276	rs6277	rs1800497
Polymorphism	1347 G>A	957C>T	TaqIA
Location	3'UTR	Exon 7 (synonymous mutation)	AnKK1 gene Exon 8 (previously reported as located in <i>DRD2</i>)
Minor allele frequency	0.484 *	0.273 *	0.296 *
Phenotype	Decreased D ₂ R function	Decreased D ₂ R mRNA stability	Decreased D ₂ R protein

* from <http://www.ncbi.nlm.nih.gov/projects/SNP/>