Role of Collectrin, an ACE2 Homologue, in Blood Pressure Homeostasis

Pei-Lun Chu, MD, PhD[1] and **Thu H. Le, MD**[1]

[1]University of Virginia Health System, Division of Nephrology, Department of Medicine, University of Virginia, Charlottesville, VA22908, PO Box 800133

Abstract

Collectrin (*Tmem27*) is a transmembrane glycoprotein that is highly expressed in the kidney and vascular endothelium. It is a homolog of the angiotensin converting enzyme 2 (ACE2) but harbors no catalytic domain. In the extravascular tissues of the kidney, collectrin is localized to the proximal tubule and collecting duct. Collectrin-deficient mice are featured with hypertension and exaggerated salt sensitivity. These phenotypes are associated with impaired uptake of the nitric oxide precursor L-arginine and the expression of its amino acid transporters, CAT-1 and y+LAT1, in endothelial cells. In addition, collectrin-deficient mice display decreased dimerization of nitric oxide synthase and decreased nitric oxide synthesis, but enhanced superoxide generation, suggesting that deletion of collectrin leads to a state of nitric oxide synthase uncoupling. These findings suggest collectrin plays a protective role against hypertension. The collectrin knockout mouse represents a unique model for hypertension research. Furthermore, collectrin may serve as a novel therapeutic target in the treatment of hypertension.

Keywords

Collectrin (*Tmem27*); Nitric oxide (NO); Endothelial nitric oxide synthase (eNOS); Pressure natriuresis; Oxidative stress; Hypertension

Introduction

Hypertension is a major risk factor for cardiovascular and renal diseases worldwide. Its pathogenesis is complex, and involves several key pathways, including the sympathetic adrenergic system, renin-angiotensin-aldosterone System (RAAS), and nitric oxide (NO) and superoxide ($O_2\bullet-$) balance. While several pathways are commonly targeted for treatment of hypertension, these existing therapies are only partially effective [1]. Currently, no treatment modality targeting the NO and/or $O_2\bullet-$ pathways has been established

Corresponding author: Thu H. Le, MD, University of Virginia Health System, Division of Nephrology, Department of Medicine, University of Virginia. Charlottesville, VA22908, PO Box 800133, Tel: 434-982-1063, THL4T@virginia.edu.

Chu and Le   Page 2

clinically for the treatment of hypertension. Recently, a novel gene, collectrin (*Tmem27*), has been identified as a key regulator of arterial pressure, which may modulate the balance of NO and/or $O_2 \bullet -$. This review will summarize current knowledge about collectrin and its role in blood pressure homeostasis.

Collectrin is a member of the Angiotensin Converting Enzyme Family

Collectrin gene (*Tmem27*) encodes a 222-amino acid transmembrane glycoprotein that shares 47.8% homology with angiotensin converting enzyme 2 (ACE2) [2]. Both ACE2 and collectrin are located on the X chromosome. By genomic sequence alignment, ACE2 has been proposed as a chimeric duplication of ACE and collectrin [3]. However, unlike ACE and ACE2, collectrin lacks enzymatic activity because of the absence of an active dipeptidyl carboxypeptidase catalytic domain [2]. Collectrin is highly conserved among species, sharing >80% identity between mouse, rat, and human [2]. Gene expression database and published studies demonstrate that collectrin is most abundantly expressed in the kidney [2]. The renal expression of collectrin is upregulated during salt-sensitive hypertension (SSH) [4] and after 5/6 nephrectomy [5]. Taken together, these observations raised the question whether collectrin plays a role in blood pressure regulation.

Function of Collectrin may be tissue specific

During mouse kidney development, collectrin expression is first detected at the ureteric bud branches on day 13 of gestation [2], followed by the *apical brush border* of proximal tubule [6], specifically in the S1 segment [7], as well as collecting duct [2]. In addition to the kidney, collectrin is highly expressed in pancreatic β-islet cells [8, 9], followed by intestine, liver, heart, as well as stomach to a lesser extent [10]. Importantly, collectrin is also expressed in the vascular endothelium of conduit and resistance vessels [11]. Because of its varied localization, collectrin may have distinct effects specific to its tissue localization. Of significance, collectrin is a downstream target of the hepatocyte nuclear factor-1α (HNF-1α) [8, 9], the mutations of which account for up to 73% of maturity onset diabetes of the young (MODY) [12, 13]. The deletion of HNF-1α in the mouse leads to Fanconi syndrome that is featured with polyuria, glucosuria, phosphaturia, and aminoaciduria [14]. We and others reported that deletion of collectrin in the mouse results in severe generalized urinary amino acid wasting due to reduced expression of neutral and cationic amino acid transporters in the plasma membrane of the proximal tubule brush border [6, 10]. Collectrin knockout mice also display polyuria, likely from a urinary concentrating defect, but the other features of Fanconi syndrome are absent [6, 10]. It is likely that collectrin acts as a chaperone for the trafficking of amino acid transporters in the proximal tubule [6]. In the vascular endothelium described in detail below, collectrin influences the uptake of L-Arginine (L-Arg). In the pancreas, gene overexpression and deletion studies in the mouse reveal that collectrin is involved in insulin secretion [8] and islet cell mass [9]. Whether there is a common pathway through which collectrin mediates its biological effect in different tissues remains to be determined. It is possible that a common pathway may be through its binding with the SNARE complex. In the colleting duct, collectrin interacts with snapin, SNAP-23, syntaxin-4 and VAMP-2 [4]. In the pancreas, collectrin facilitates SNARE complex formation through interacting with snapin, a synaptosomal-associated protein [8].

Curr Hypertens Rep. Author manuscript; available in PMC 2015 November 01.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

In addition to the full-length 222-amino acid collectrin, a cleaved soluble N-terminal (extracellular) fragment of collectrin has also been identified in both the pancreas [15] and the kidney [16]. The cleavage is mediated by beta site amyloid precursor protein cleaving enzyme 2 (*Bace2*) [15]. Currently, the biological function of the cleaved collectrin is still unknown.

Collectrin Links Amino Acid Transport with Blood Pressure Regulation

Its homology to ACE2 and its upregulation in the kidney during high salt feeding and after 5/6 nephrectomy raised the central question whether collectrin might play a role in blood pressure homeostasis. It was thus surprising that the key phenotype observed in collectrin knockout (KO) mice is massive urinary amino acid wasting [6, 10]. In our initial characterization of collectrin KO mice, on a mixed genetic background of 129/SvEv × C57BL/6J, we did not detect a statistically significant difference in arterial pressure compared to wild-type (WT) mice [4]. Due to the possibility of genetic background masking any arterial pressure effect commonly observed in many mouse models of cardiovascular diseases and hypertension (HTN), including *Ace2* [17], we therefore backcrossed the collectrin null mutation on the 129/SvEV background for > 12 generations to generate inbred 129/SvEv KO mice. This 129 strain is more susceptible to the development of HTN and kidney disease than the C57BL/6J [18, 19]. Furthermore, it is well recognized that the 129/SvEv strain is a salt-sensitive strain [20-22], and collectrin's expression is upregulated during SSH in rat models [4]. On this salt sensitive background, we found that collectrin KO mice have hypertension at baseline and augmented salt sensitivity that is associated with impaired pressure natriuresis [11]. These phenotypes are associated with increased $O_2\bullet-$ generation and decreased NO production in the kidney *in vivo*, and impaired endothelium-dependent relaxation of resistance arteries *ex vivo*. These effects resulting from deletion of collectrin are independent of activation of the RAS, since there were no detectable significant differences in mRNA levels of ACE-2, Mas receptor [11], and AT_{1A} receptor (unpublished data). There was even a trend towards lower plasma renin activity in KO mice [11]. These observations suggest that collectrin KO mice have impaired arterial pressure control as a result of abnormal NO synthesis and $O_2\bullet-$ generation, an imbalance that may involve the uncoupling of nitric oxide synthase (NOS). Furthermore, our findings raised a key question whether there is a causal link between amino acid transport defect and blood pressure regulation in collectrin KO mice.

Collectrin and the L-arginine-nitric oxide pathway

The NO signaling pathway is recognized as a key second messenger systems involved in the regulation of vascular tone and arterial pressure [23]. NO is synthesized from L-Arg, a semiessential cationic AA, through a reaction catalyzed by NOS in the presence of the cofactor tetrahydrobiopterin (BH4) [24]. There are three NOS isoforms: endothelial (eNOS) and neuronal (nNOS) isoforms are constitutively expressed, and the inducible isoform (iNOS) mainly expressed by activated inflammatory cells. Both eNOS and nNOS are only fully functional in a dimeric form, and the stabilization of their dimeric forms is dependent on several factors, including the availability of L-Arg substrate and BH4 co-factor, and S-nitrosylation [25]. In the absence of L-Arg and/or BH4, NOS cannot generate NO, but is

capable of generating $O_2\bullet-$, a phenomenon commonly referred to as "<u>uncoupling</u>" [25, 26] that can lead to the development of endothelial dysfunction (ED) or impaired ability of the vascular endothelium to stimulate vasodilation, and hence the maintenance of HTN. To evaluate for possible altered eNOS expression in collectrin KO mice, we compared baseline levels of eNOS dimer (active eNOS) to monomer (inactive eNOS) in the aorta, kidney cortex and medulla from WT and collectrin KO mice. By protein analysis in all three tissues, expression of eNOS dimer was significantly lower in KO mice compared to WT [11]. In addition, nNOS dimer levels were also significantly lower in renal tissue in collectrin KO mice (unpublished data). Our data suggest a state of NOS uncoupling that favors increased $O_2\bullet-$ production in collectrin KO mice.

The altered balance of NO and $O_2\bullet-$, diminished NOS dimerization or "uncoupling", and the known functional property of collectrin as a chaperone of amino acid transporter in the proximal tubule raised the possibility that the central defect in blood pressure homeostasis could be due to impaired cellular uptake of L-Arg in collectrin KO mice. Indeed, we showed that primary endothelial cells from collectrin KO mice were shown to have decreased [^{3}H]L-Arg uptake [11]. In addition, overexpression of collectrin in primary human coronary endothelial cells resulted in increased [^{3}H]L-Arg uptake [11].

There is emerging evidence that there are several different L-Arg sources or pools for NO synthesis by NOS: 1) plasma membrane transport of L-Arg from the extracellular space [27], 2) intracellular L-citrulline to L-Arg recycling [28], and 3) lysosomal and proteasomal protein degradation that in turn also generates the L-Arg analogue asymmetric dimethyl-L-Arg (ADMA), an endogenous inhibitor of NOS [29]. Certain pathophysiologic conditions may determine which L-Arg pool is the rate limiting source [29]. In several cell types, including the endothelium and renal epithelium, synthesis of NO requires the <u>*de novo*</u> import of extracellular L-Arg [30-32]. Under normal physiologic conditions, intracellular concentration of L-Arg is in far excess (20 to 760 fold) of the Km for both eNOS and nNOS, and normal plasma L-Arg concentration in humans and animals ranges 5 to 10 fold the Km for NOS [33, 34]. However, further elevation of extracellular L-Arg levels by L-Arg administration directly enhances endothelial NO production. The dependence of endogenous NO generation on the uptake of extracellular L-Arg has led to the concept of "<u>L-arginine paradox</u>" [33, 34]. It follows that a defect in the import of extracellular L-Arg can cause endothelial dysfunction and HTN. In support, Schaich et al. demonstrated that impaired L-Arg transport, both *in vitro* and in *vivo*, occurs in patients with essential HTN, as well as in normotensive individuals with a positive family history of essential HTN [35]. The effect of L-Arg administration may be mediated both by endothelial and renal epithelial cells. In this regard, hypertensive humans and animals respond to L-Arg administration with reduced arterial pressure and improved endothelial vasodilator function [36, 37]. Renal medullary interstitial infusion of L-Arg to Dahl salt-sensitive rats prevents the development of HTN during high-salt feeding [38]. We reported that treatment with L-Arg partially lowered blood pressure in collectrin KO mice [11]. Taken together, these data suggest that collectrin may also chaperone the proper localization and/or function of amino acid transporters for L-Arg.

L-Arg transport into cells is mediated by different classes of amino acid transporters that are defined by their ion dependency, substrate specificity, and relative affinity [39, 34]. In

endothelial cells (ECs), the system y^+, a sodium-independent system, accounts for ~60% of L-Arg transport, and system y^+L, a sodium-dependent system, accounts for ~40% of L-Arg transport [40, 41, 30]. System y^+ selectively mediates the cellular transport of cationic amino acids, including L-Arg, whereas system y^+L transports both cationic and neutral amino acids [30, 32]. Cloning studies have identified at least 3 cationic transporters in system y^+, namely, the cationic amino acid transporter (CAT) proteins: CAT1, CAT2A and CAT2B isoforms, and CAT3 [39]. System y^+L is a glycoprotein-associated amino acid heterodimeric transporter whose heavy chain is the glycoprotein 4F2hc/CD98, and the hydrophobic "light chain", y^+LAT1 or y^+LAT2, is responsible for the recognition and operational features of the transport process [42]. Both y^+ and y^+L systems are also expressed in renal endothelial and epithelial cells, and play an important role in influencing L-Arg uptake, NO production, medullary blood flow, and arterial pressure [31, 43, 44, 38].

Analogous amino acid transport systems, encoded by different genes, exist in epithelial cells such as the kidney and intestine. In the kidney, in addition to y^+ and the y^+L systems which have been well described in the renal endothelium and inner medullary CD [43, 45], the broad-scope transporters also have been well characterized: B^0, system 1, is sodium-dependent and is a low affinity transporter for almost all neutral amino acids [46] but also has preference for the cationic substrates lysine and arginine [47, 48]; $b^{0,+}$, system 2, is sodium-independent, and is an antiporter that takes up cationic amino acid in exchange for neutral amino acids [46]. These two systems are expressed along the brush border of the proximal tubule [48]. We and others reported that collectrin KO mice have severe aminoaciduria due to decreased protein expression of B^0AT1 (B^0 system), and $b^{0,+}AT/rBAT$ heteromeric complex ($b^{0,+}$ system) in the plasma membrane fractions of the renal proximal tubule [6]. Furthermore, the expression of the y^+L system (y^+LAT1 and y^+LAT2) in kidney plasma membrane fractions are also decreased in collectrin KO mice [11]. Moreover, through gene knockdown and overexpression studies, we found that collectrin directly mediates the plasma membrane expression of the y^+ and y^+L transporter systems in ECs [11]. Collectively, our studies suggest that collectrin is a genetic factor that regulates arterial pressure and salt-sensitivity, likely by modulating the L-Arg/NO/O_2•– pathway through its role in regulating expression of amino acid transporters and cellular L-Arg uptake.

Our finding provides a corollary to a published report that a patient with compound heterozygosity for 2 mutations in the *SLC7A7* gene that encodes the y+LAT1 transporter exhibited vascular endothelial dysfunction that was corrected with L-Arg infusion [49]. Similarly, the diminished CAT1 (*SLC7A1*) expression caused by a single nucleotide polymorphism (SNP) in the 3′ UTR of *SLC7A1* has been linked to hypertension and endothelial dysfunction [50]. Thus, collectrin may modulate endothelial function and blood pressure through its central role in chaperoning the L-Arg transporters CAT1 and y+L. However, while decreased L-Arg availability due to impaired uptake may be the cause for decreased eNOS dimerization in the KO mice, we cannot rule out the possibility that collectrin may influence eNOS dimerization independently of its effect on L-Arg uptake by interacting with other proteins that play a role in stabilizing eNOS. For example, caveolae, which are important in determining eNOS intracellular localization and activity [25], contain some members of the SNARE complex [51] with which collectrin binds and interacts [8, 4].

Although the functional role of collectrin in L-Arg uptake has been delineated primarily via endothelial cells *in vitro*, collectrin was initially identified in the collecting duct [2]. We have since confirmed this finding [6]; and, specifically, while collectrin is expressed on the brush border of the proximal tubule, it is localized to the basolateral side of the collecting duct. Of particular relevance, the cellular uptake of L-Arg by CAT1 in the renal medulla is critical for NO production, maintenance of medullary blood flow, and regulation of Na^+ excretion and blood pressure [31, 43]. The source of NO in the renal medulla could be from either eNOS and/or nNOS. Neuronal NOS is highly expressed in the collecting duct, and is significantly increased in the early phase of high salt intake, where it is thought to generate NO as an adaptive response to salt intake [10]. The renal expression of collectrin is also increased during high salt diet [4], suggesting that collectrin is involved in this pathway. We queried whether loss of collectrin also affects nNOS dimerization and its activity. Western analysis of renal medullary tissue revealed that nNOS dimerization is significantly diminished in KO mice compared to WT, despite no changes in mRNA levels (unpublished data), consistent with our findings for eNOS. This suggests that collectrin influences the dimerization of both eNOS and nNOS post-transcriptionally, likely through its role mediating L-Arg uptake.

Data have emerged that collecting duct-derived NO plays a key role in the regulation of arterial pressure and sodium excretion. In particular, Kohan and associates demonstrated that the effect of collecting duct-derived endothelin in arterial pressure regulation and Na^+ excretion is mediated through NO [52]. Recently, Pollock and associates showed that collecting duct nNOS regulates arterial pressure and fluid-electrolyte homeostasis [53]. Our findings that collectrin KO mice have impaired pressure-natriuresis relationship that is associated with decreased nNOS activity/dimerization is consistent with the notion that collectrin in the collecting duct regulates arterial pressure through its effect on nNOS and Na^+ excretion.

Future Considerations

Guyton and colleagues hypothesized that the kidney's substantial capacity for Na^+ excretion provides a compensatory system of virtually infinite gain to oppose processes causing elevation in arterial pressure, including increases in peripheral vascular resistance (PVR). It follows that, in collectrin KO mice, even if the initial cause of HTN is due to increased PVR from reduced vasorelaxation, a defect in renal excretory function would be a pre-requisite for the sustained chronic increase in arterial pressure. The defect in Na^+ handling and altered pressure-natriuresis observed during high salt diet feeding in collectrin KO mice suggests that altered kidney function could result from a defect in renal epithelial function and/or renal hemodynamics. Future efforts including generation of mouse lines in which collectrin is deleted specifically in the endothelium, proximal tubule and collecting duct, will be crucial to determine the relative contribution of collectrin in each of these tissues to blood pressure homeostasis.

The dependence on a proper genetic background for the measureable effect of collectrin on arterial pressure suggests that there is a genetic modifier that facilitates the development of

hypertension in the context of loss of function of collectrin. Gene mapping studies using 129/SvEv × C57BL/6J intercrosses may enable identification of this genetic modifier.

Collectrin is located on the X chromosome, and is harbored by a locus that modulates blood pressure in female rats in the Sabra rat model of SSH [54], raising the possibility that collectrin may be a genetic factor in HTN. Although it is not known whether there are any loci on the X chromosome that contribute to HTN in humans - since genome wide association studies (GWAS) have largely excluded X chromosome variants from analyses - there are known variants altering mRNA transcript structures (splicing, frameshift and stop gained SNPs) and amino acid composition (missense/non-synonymous SNPs) in the human collectrin gene (http://ensembl.org/Homo_sapiens/Gene/Variation_Gene/Table?db=core;g=ENSG00000147003;r=X:15645441-15683154;t=ENST00000380342). Our eventual goal would be test for the association of these human SNPs in a cohort of patients with hypertension and salt-sensitivity.

Potential Clinical Application

Collectively, the evidence demonstrates that collectrin has a protective role in HTN and salt sensitivity, at least by maintaining a balance between NO and $O_2\bullet-$, likely through its central function in chaperoning L-Arg transport in endothelial cells and possibly in renal epithelial cells. Theoretically, modulation of the expression of collectrin, either by increasing its expression or decreasing its metabolism, might serve as a novel therapeutic approach in the treatment of HTN. In this regard, the beta site amyloid precursor protein cleaving enzyme 2 (*Bace2*), a transmembrane aspartic protease that is expressed in multiple tissues including the pancreas and kidney [16], might be a therapeutic target of this pathway. It has been demonstrated that *Bace2* is able to cleave collectrin and releases the soluble N-terminal fragment of collectrin [15], resulting in rapid degradation of the remaining membrane-bound C-terminal collectrin fragment [9]. The deletion of *Bace2* causes an increase in collectrin protein expression [15]. Currently, the cleavage of collectrin has only been reported in beta cells in the pancreas [15] and primary proximal tubular cells isolated from mouse kidneys [55], but the biological significance of the cleavage as well as the soluble N-terminal fragment are unknown.

Pharmacological inhibition of *Bace2* is able to increase β-islet cell mass, increase insulin secretion and improve glycemic control in obesity mouse models (*ob/ob* and C57BKS$^{db/db}$) [15]. These effects are thought to be through inhibition of the cleavage of collectrin. However, whether *Bace2* inhibitor is able to modulate blood pressure remains to be determined. Currently, the available *Bace2* inhibitors are not selective and also exhibit off-target effect on other BACE enzymes [56].

An alternative therapeutic approach might involve direct modification of collectrin dimerization, deglycosylation, plasma membrane depletion and its extracellular Phe-Phe motif to enable collectrin to be resistant to cleavage of *Bace2* [56]. However, these modifications might inevitably affect the physiological characteristics of collectrin such as its plasma membrane localization [56]. Future research focusing on selective *Bace2*

inhibition or direct collectrin stabilization may yield novel therapeutic strategies for the treatment of HTN, particularly in conditions where the balance of NO and $O_2\bullet-$ is altered.

Conclusion

Arguably a distant member of the Renin-Angiotensin System, collectrin is a highly conserved gene that encodes a 222-amino acid transmembrane glycoprotein that is a member of the angiotensin-converting enzyme (ACE) family, sharing ~50% homology with ACE2, a carboxypeptidase that directly converts the vasoconstrictive peptide angiotensin II (Ang II) to the vasodilatory peptide angiotensin 1-7. Unlike ACE2, collectrin lacks a catalytic domain. In the context of conceptual advancement, the newly discovered role of collectrin as a mediator of L-Arg uptake in endothelial cells directly links L-Arg uptake together with "eNOS uncoupling" in blood pressure regulation, by modulating NO and $O_2\bullet-$ balance. These findings may challenge the existing paradigm of the Renin-Angiotensin System as a simple cascade of enzymatic events. Collectrin may also influence other physiologic and pathological conditions that are dependent on nitric oxide and/or superoxide signaling.

References

Papers of particular interest, published recently, have been highlighted as:

* of importance.

1. Chow CK, Teo KK, Rangarajan S, Islam S, Gupta R, Avezum A, et al. Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA. 2013; 310(9):959–68. doi:10.1001/jama.2013.184182. [PubMed: 24002282]

2. Zhang H, Wada J, Hida K, Tsuchiyama Y, Hiragushi K, Shikata K, et al. Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. J Biol Chem. 2001; 276(20):17132–9. doi:10.1074/jbc.M006723200M006723200 [pii]. [PubMed: 11278314]

3. Mount DB. Collectrin and the kidney. Curr Opin Nephrol Hypertens. 2007; 16(5):427–9. doi:10.1097/MNH.0b013e3282e9acc5. [PubMed: 17693757]

4. Yasuhara A, Wada J, Malakauskas SM, Zhang Y, Eguchi J, Nakatsuka A, et al. Collectrin is involved in the development of salt-sensitive hypertension by facilitating the membrane trafficking of apical membrane proteins via interaction with soluble N-ethylmaleiamide-sensitive factor attachment protein receptor complex. Circulation. 2008; 118(21):2146–55. doi:CIRCULATIONAHA.108.787259 [pii] 10.1161/CIRCULATIONAHA.108.787259. [PubMed: 18981302]

5. Zhang H, Wada J, Kanwar YS, Tsuchiyama Y, Hiragushi K, Hida K, et al. Screening for genes up-regulated in 5/6 nephrectomized mouse kidney. Kidney Int. 1999; 56(2):549–58. doi:kid561 [pii] 10.1046/j.1523-1755.1999.00561.x. [PubMed: 10432394]

6. Malakauskas SM, Quan H, Fields TA, McCall SJ, Yu MJ, Kourany WM, et al. Aminoaciduria and altered renal expression of luminal amino acid transporters in mice lacking novel gene collectrin. Am J Physiol Renal Physiol. 2007; 292(2):F533–44. doi:00325.2006 [pii] 10.1152/ajprenal.00325.2006. [PubMed: 16985211]

7. McCoy KE, Zhou X, Vize PD. Collectrin/tmem27 is expressed at high levels in all segments of the developing Xenopus pronephric nephron and in the Wolffian duct. Gene expression patterns: GEP. 2008; 8(4):271–4. doi:10.1016/j.gep.2007.12.002. [PubMed: 18226983]

8. Fukui K, Yang Q, Cao Y, Takahashi N, Hatakeyama H, Wang H, et al. The HNF-1 target collectrin controls insulin exocytosis by SNARE complex formation. Cell Metab. 2005; 2(6):373–84. doi:S1550-4131(05)00339-6 [pii] 10.1016/j.cmet.2005.11.003. [PubMed: 16330323]

9. Akpinar P, Kuwajima S, Krutzfeldt J, Stoffel M. Tmem27: a cleaved and shed plasma membrane protein that stimulates pancreatic beta cell proliferation. Cell Metab. 2005; 2(6):385–97. doi:S1550-4131(05)00335-9 [pii] 10.1016/j.cmet.2005.11.001. [PubMed: 16330324]

10. Danilczyk U, Sarao R, Remy C, Benabbas C, Stange G, Richter A, et al. Essential role for collectrin in renal amino acid transport. Nature. 2006; 444(7122):1088–91. doi:10.1038/nature05475. [PubMed: 17167413]

11•. Cechova S, Zeng Q, Billaud M, Mutchler S, Rudy CK, Straub AC, et al. Loss of collectrin, an angiotensin-converting enzyme 2 homolog, uncouples endothelial nitric oxide synthase and causes hypertension and vascular dysfunction. Circulation. 2013; 128(16):1770–80. doi:10.1161/CIRCULATIONAHA.113.003301. [PubMed: 24048198] This study established the role of collectrin in blood pressure regulation. Furthermore, the study links amino acid transport defect with "L-arginine paradox", endothelial dysfunction and hypertension.

12. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, et al. Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). Nature. 1996; 384(6608):455–8. doi:10.1038/384455a0. [PubMed: 8945470]

13. Shih DQ, Stoffel M. Molecular etiologies of MODY and other early-onset forms of diabetes. Current diabetes reports. 2002; 2(2):125–34. [PubMed: 12643132]

14. Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, et al. Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell. 1996; 84(4):575–85. [PubMed: 8598044]

15•. Esterhazy D, Stutzer I, Wang H, Rechsteiner MP, Beauchamp J, Dobeli H, et al. Bace2 is a beta cell-enriched protease that regulates pancreatic beta cell function and mass. Cell Metab. 2011; 14(3):365–77. doi:10.1016/j.cmet.2011.06.018. [PubMed: 21907142] This report identified the beta site amyloid precursor protein cleaving enzyme 2 (Bace2) as the sheddase of collectrin, suggesting that Bace2 may be a therapeutic target in conditions in which increased expression of collectrin may be desireable for potential beneficial effects such as salt sensitive hypertension.

16. Bennett BD, Babu-Khan S, Loeloff R, Louis JC, Curran E, Citron M, et al. Expression analysis of BACE2 in brain and peripheral tissues. J Biol Chem. 2000; 275(27):20647–51. doi:10.1074/jbc.M002688200. [PubMed: 10749877]

17. Gurley SB, Allred A, Le TH, Griffiths R, Mao L, Philip N, et al. Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. J Clin Invest. 2006; 116(8):2218–25. doi:10.1172/JCI16980. [PubMed: 16878172]

18. Hartner A, Cordasic N, Klanke B, Veelken R, Hilgers KF. Strain differences in the development of hypertension and glomerular lesions induced by deoxycorticosterone acetate salt in mice. Nephrol Dial Transplant. 2003; 18(10):1999–2004. doi:10.1093/ndt/gfg299 18/10/1999 [pii]. [PubMed: 13679473]

19. Salzler HR, Griffiths R, Ruiz P, Chi L, Frey C, Marchuk DA, et al. Hypertension and albuminuria in chronic kidney disease mapped to a mouse chromosome 11 locus. Kidney Int. 2007; 72(10): 1226–32. [PubMed: 17851470]

20. Francois H, Athirakul K, Howell D, Dash R, Mao L, Kim HS, et al. Prostacyclin protects against elevated blood pressure and cardiac fibrosis. Cell Metab. 2005; 2(3):201–7. doi:S1550-4131(05)00233-0 [pii] 10.1016/j.cmet.2005.08.005. [PubMed: 16154102]

21. Ryan MJ, Didion SP, Davis DR, Faraci FM, Sigmund CD. Endothelial dysfunction and blood pressure variability in selected inbred mouse strains. Arterioscler Thromb Vasc Biol. 2002; 22(1): 42–8. [PubMed: 11788459]

22. Wang Q, Hummler E, Nussberger J, Clement S, Gabbiani G, Brunner HR, et al. Blood pressure, cardiac, and renal responses to salt and deoxycorticosterone acetate in mice: role of Renin genes. J Am Soc Nephrol. 2002; 13(6):1509–16. [PubMed: 12039980]

23. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem J. 1994; 298(Pt 2):249–58. [PubMed: 7510950]

24. Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, et al. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. J Clin Invest. 2005; 115(5):1221–31. [PubMed: 15841206]

25. Balligand JL, Feron O, Dessy C. eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. Physiol Rev. 2009; 89(2):481–534. doi:89/2/481 [pii] 10.1152/physrev.00042.2007. [PubMed: 19342613]

26. Verhaar MC, Westerweel PE, van Zonneveld AJ, Rabelink TJ. Free radical production by dysfunctional eNOS. Heart. 2004; 90(5):494–5. [PubMed: 15084540]

27. Closs EI, Scheld JS, Sharafi M, Forstermann U. Substrate supply for nitric-oxide synthase in macrophages and endothelial cells: role of cationic amino acid transporters. Mol Pharmacol. 2000; 57(1):68–74. [PubMed: 10617680]

28. Hecker M, Sessa WC, Harris HJ, Anggard EE, Vane JR. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine. Proc Natl Acad Sci U S A. 1990; 87(21):8612–6. [PubMed: 2236071]

29. Simon A, Plies L, Habermeier A, Martine U, Reining M, Closs EI. Role of Neutral Amino Acid Transport and Protein Breakdown for Substrate Supply of Nitric Oxide Synthase in Human Endothelial Cells. Circ Res. 2003; 93(9):813–20. doi:10.1161/01.res.0000097761.19223.0d. [PubMed: 14512444]

30. Arancibia-Garavilla Y, Toledo F, Casanello P, Sobrevia L. Nitric oxide synthesis requires activity of the cationic and neutral amino acid transport system y+L in human umbilical vein endothelium. Exp Physiol. 2003; 88(6):699–710. doi:EPH_2647 [pii]. [PubMed: 14603368]

31. Kakoki M, Kim HS, Arendshorst WJ, Mattson DL. L-Arginine uptake affects nitric oxide production and blood flow in the renal medulla. Am J Physiol Regul Integr Comp Physiol. 2004; 287(6):R1478–85. doi:10.1152/ajpregu.00386.2004 00386.2004 [pii]. [PubMed: 15319219]

32. Kakoki M, Kim HS, Edgell CJ, Maeda N, Smithies O, Mattson DL. Amino acids as modulators of endothelium-derived nitric oxide. Am J Physiol Renal Physiol. 2006; 291(2):F297–304. doi:10.1152/ajprenal.00417.2005. [PubMed: 16571593]

33. Rajapakse NW, Mattson DL. Role of L-arginine in nitric oxide production in health and hypertension. Clin Exp Pharmacol Physiol. 2009; 36(3):249–55. doi:10.1111/j.1440-1681.2008.05123.x. [PubMed: 19076168]

34. Yang Z, Kaye DM. Endothelial dysfunction and impaired L-arginine transport in hypertension and genetically predisposed normotensive subjects. Trends Cardiovasc Med. 2006; 16(4):118–24. doi:10.1016/j.tcm.2006.02.003. [PubMed: 16713534]

35. Schlaich MP, Parnell MM, Ahlers BA, Finch S, Marshall T, Zhang WZ, et al. Impaired L-arginine transport and endothelial function in hypertensive and genetically predisposed normotensive subjects. Circulation. 2004; 110(24):3680–6. doi:01.CIR.0000149748.79945.52 [pii] 10.1161/01.CIR.0000149748.79945.52. [PubMed: 15569830]

36. Chen PY, Sanders PW. Role of nitric oxide synthesis in salt-sensitive hypertension in Dahl/Rapp rats. Hypertension. 1993; 22(6):812–8. [PubMed: 7503951]

37. Siani A, Pagano E, Iacone R, Iacoviello L, Scopacasa F, Strazzullo P. Blood pressure and metabolic changes during dietary L-arginine supplementation in humans. Am J Hypertens. 2000; 13(5 Pt 1):547–51. doi:S0895-7061(99)00233-2 [pii]. [PubMed: 10826408]

38. Pallone TL, Mattson DL. Role of nitric oxide in regulation of the renal medulla in normal and hypertensive kidneys. Curr Opin Nephrol Hypertens. 2002; 11(1):93–8. [PubMed: 11753093]

39. Dye JF, Vause S, Johnston T, Clark P, Firth JA, D'Souza SW, et al. Characterization of cationic amino acid transporters and expression of endothelial nitric oxide synthase in human placental microvascular endothelial cells. FASEB J. 2004; 18(1):125–7. doi:10.1096/fj.02-0916fje 02-0916fje [pii]. [PubMed: 14597568]

40. Greene B, Pacitti AJ, Souba WW. Characterization of L-arginine transport by pulmonary artery endothelial cells. Am J Physiol. 1993; 264(4 Pt 1):L351–6. [PubMed: 8476065]

41. Kilberg MS, Stevens BR, Novak DA. Recent advances in mammalian amino acid transport. Annu Rev Nutr. 1993; 13:137–65. doi:10.1146/annurev.nu.13.070193.001033. [PubMed: 8369142]

42. Verrey F, Jack DL, Paulsen IT, Saier MH Jr. Pfeiffer R. New glycoprotein-associated amino acid transporters. J Membr Biol. 1999; 172(3):181–92. doi:JMEMB124 [pii]. [PubMed: 10568788]

43. Kakoki M, Wang W, Mattson DL. Cationic amino acid transport in the renal medulla and blood pressure regulation. Hypertension. 2002; 39(2):287–92. [PubMed: 11847199]

44. Mattson DL, Meister CJ. Renal cortical and medullary blood flow responses to L-NAME and ANG II in wild-type, nNOS null mutant, and eNOS null mutant mice. Am J Physiol Regul Integr Comp Physiol. 2005; 289(4):R991–7. doi:00207.2005 [pii] 10.1152/ajpregu.00207.2005. [PubMed: 15961532]

45. Verrey F, Ristic Z, Romeo E, Ramadan T, Makrides V, Dave MH, et al. Novel renal amino acid transporters. Annu Rev Physiol. 2005; 67:557–72. [PubMed: 15709970]

46. Broer S. Apical Transporters for Neutral Amino Acids: Physiology and Pathophysiology. Physiology. 2008; 23:95–103. [PubMed: 18400692]

47. Dall'Asta V, Bussolati O, Sala R, Rotoli BM, Sebastio G, Sperandeo MP, et al. Arginine transport through system y(+)L in cultured human fibroblasts: normal phenotype of cells from LPI subjects. Am J Physiol Cell Physiol. 2000; 279(6):C1829–37. [PubMed: 11078698]

48. Deves R, Boyd CA. Transporters for cationic amino acids in animal cells, discovery, structure, and function. Physiol Rev. 1998; 78:487–545. [PubMed: 9562037]

49. Kamada Y, Nagaretani H, Tamura S, Ohama T, Maruyama T, Hiraoka H, et al. Vascular endothelial dysfunction resulting from l-arginine deficiency in a patient with lysinuric protein intolerance. The Journal of Clinical Investigation. 2001; 108(5):717–24. [PubMed: 11544277]

50. Yang Z, Venardos K, Jones E, Morris BJ, Chin-Dusting J, Kaye DM. Identification of a novel polymorphism in the 3′UTR of the L-arginine transporter gene SLC7A1: contribution to hypertension and endothelial dysfunction. Circulation. 2007; 115(10):1269–74. doi:CIRCULATIONAHA.106.665836 [pii] 10.1161/CIRCULATIONAHA.106.665836. [PubMed: 17325243]

51. Schnitzer JE, Liu J, Oh P. Endothelial caveolae have the molecular transport machinery for vesicle budding, docking, and fusion including VAMP, NSF, SNAP, annexins, and GTPases. J Biol Chem. 1995; 270(24):14399–404. [PubMed: 7782301]

52. Schneider MP, Ge Y, Pollock DM, Pollock JS, Kohan DE. Collecting Duct-Derived Endothelin Regulates Arterial Pressure and Na Excretion via Nitric Oxide. Hypertension. 2008; 51(6):1605–10. doi:10.1161/hypertensionaha.107.108126. [PubMed: 18391099]

53. Hyndman KA, Boesen EI, Elmarakby AA, Brands MW, Huang P, Kohan DE, et al. Renal collecting duct NOS1 maintains fluid-electrolyte homeostasis and blood pressure. Hypertension. 2013; 62(1):91–8. doi:HYPERTENSIONAHA.113.01291 [pii] 10.1161/HYPERTENSIONAHA. 113.01291. [PubMed: 23608660]

54. Yagil C, Sapojnikov M, Kreutz R, Zurcher H, Ganten D, Yagil Y. Role of Chromosome X in the Sabra Rat Model of Salt-Sensitive Hypertension. Hypertension. 1999; 33(1):261–5. [PubMed: 9931114]

55. Altirriba J, Gasa R, Casas S, Ramirez-Bajo MJ, Ros S, Gutierrez-Dalmau A, et al. The role of transmembrane protein 27 (TMEM27) in islet physiology and its potential use as a beta cell mass biomarker. Diabetologia. 2010; 53(7):1406–14. doi:10.1007/s00125-010-1728-6. [PubMed: 20386877]

56•. Esterhazy D, Akpinar P, Stoffel M. Tmem27 dimerization, deglycosylation, plasma membrane depletion, and the extracellular Phe-Phe motif are negative regulators of cleavage by Bace2. Biol Chem. 2012; 393(6):473–84. doi:10.1515/hsz-2012-0104/j/bchm.2012.393.issue-6/ hsz-2012-0104/hsz-2012-0104.xml [pii]. [PubMed: 22628310] This study identified sites of collectrin protein that determine its susceptibility to cleavage by Bace2, and may have implication for the manipulation of the stability of collectrin in certain disease states.