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Uremic toxins are conditional danger- or homeostasis-associated molecular patterns

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1. ABSTRACT

We mined novel uremic toxin (UT) metabolomics/gene databases, and analyzed the expression changes of UT receptors and UT synthases in chronic kidney disease (CKD) and cardiovascular disease (CVD). We made the following observations: 1) UTs represent only 1/80th of human serum small-molecule metabolome; 2) Some UTs are increased in CKD and CVD; 3) UTs either induce or suppress the expression of inflammatory molecules; 4) The expression of UT genes is significantly modulated in CKD patients, and coronary artery disease (CAD) patients; 5) The expression of UT genes is upregulated by caspase-1 and TNF-alpha pathways but is inhibited in regulatory T cells. These results demonstrate that UTs are selectively increased, and serve as danger signal-associated molecular patterns (DAMPs) and homeostasis-associated molecular patterns (HAMPs) that modulate inflammation. These results also show that some UT genes are upregulated in CKD and CAD via caspase-1/inflammatory cytokine pathways, rather than by purely passive accumulation.

2. INTRODUCTION

The incidence of chronic kidney disease (CKD) is increasing worldwide. Atherosclerosis-related cardiovascular disease (CVD) is a major cause of mortality in patients with CKD (1). We and others have previously shown that hyperlipidemia, along with other CVD stressors, such as hyperglycemia, hyperhomocysteinemia, and chronic kidney disease, promote atherosclerosis and vascular inflammation via several mechanisms (2–7). These mechanisms include endothelial cell (EC) activation and injury (2,8–10); mitochondrial reactive oxygen species (3); monocyte recruitment and differentiation (11,12); decreased

regulatory T cells (13–15); impaired vascular repair ability of bone marrow–derived progenitor cells (16, 17); and downregulated histone modification enzymes (18).

CKD ranges from mild CKD to end-stage renal disease (ESRD), which requires therapies such as life-long hemodialysis or kidney transplantation (19). CKD is classified into 5 stages based on glomerular filtration rate (GFR, mL/min. per 1.7.3m^2); ≥90mL/min (stage 1), 60–89mL/min (stage 2), 30–59mL/min (stage 3), 15–29mL/min (stage 4) and <15mL/min (stage 5). At stage 5, the patient develops ESRD, and requires dialysis. Tests for kidney function include creatinine clearance, creatinine levels, and blood urea nitrogen (BUN) assessment (MedlinePlus, NIH https://medlineplus.gov/kidneytests.html). CVD risk increases significantly according to the stages of CKD, ranging from 1.5.-fold in stage 2, to between 20 and 1,000-fold with ESRD (20). Indeed, CVD accounts for approximately 50% of deaths in patients receiving dialysis (21). These clinical data clearly demonstrate that CKD accelerates atherosclerosis, which along with its complications such as myocardial infarction, stroke and peripheral artery disease, are the leading cause of morbidity and mortality in the U.S., and account for 75% of all deaths from CVD (20, 22). The molecular and cellular mechanisms underlying CKD-accelerated atherosclerosis, especially the important issue of receptors in sensing uremic toxins (UTs), remain unknown (23).

It has been suggested that CKD uremic toxins (UTs), in combination with other risk factors, cause oxidative stress, low-grade inflammation with increased circulating cytokines and endothelial dysfunction (20, 23). One of the well-characterized UTs is carbamylated LDL (cLDL) (24). Urea spontaneously dissociates to form cyanate (OCN^-), which modifies proteins in a process referred to as carbamylation. The active form of cyanate, isocyanic acid, reacts irreversibly with the amino acids in apolipoprotein B, the protein component of LDL to form cLDL (24). Protein carbamylation has been found in atherosclerotic plaque and serum level of cLDL is increased significantly in patients with ESRD. In addition, cLDL, but not native LDL, has been shown to have all of the major biological effects relevant to atherosclerosis, including EC injury and dysfunction by binding to oxLDL receptor (LOX-1), increased expression of cell adhesion molecules, monocyte adhesion, and vascular smooth muscle cell (VSMC) proliferation (24–26). However, the mechanistic link between sensing UTs and vascular inflammation remains unknown.

Cellular “receptors”, which can recognize the risk factors for vascular inflammation and atherogenesis, have been intensively researched. The role of pathogen-associated molecular patterns (PAMPs) and danger signal-associated molecular patterns (DAMPs) receptors has been characterized recently as bridging innate immune sensory systems for exogenous infectious agents and endogenous metabolic dangers to initiation of inflammation (27). More than 14 groups of endogenous metabolites have been proposed to act as danger signals via various DAMP recognition receptors to promote inflammation (28, 29). The Toll-like receptors (TLRs), mainly localized in the plasma membrane, recognize a variety of conserved microbial PAMPs and metabolic DAMPs, thereby functioning as PAMP and DAMP receptors, and promote inflammatory gene transcription. As we reported previously, for inflammation-privileged tissues, such as cardiovascular tissues in which inflammasome component genes are not constitutively expressed, TLRs work in synergy with upregulated cytosol-located sensing receptor families including NLRs (NOD (nucleotide binding and oligomerization domain)-like receptors) (30). In the cytosol, nucleus and extracellular compartment as we most recently reported, these inflammasome components and pro-caspase-1 assemble into a protein complex termed inflammasome, which subsequently activates caspase-1 after recognizing endogenous DAMPs (31). In this way, TLRs mediate upregulation, activation of a range of inflammatory genes and acceleration of vascular inflammation and atherosclerosis (2,32). After recognizing a paradox that classical DAMP receptors may not be able to bind with high affinity to all of the endogenous metabolite-derived danger signals, we proposed that endogenous metabolite-derived danger signals are conditional DAMPs, which together with our newly proposed homeostasis (anti-inflammatory)-associated molecular patterns (HAMPs), may use both intrinsic receptors and classical DAMP receptors to regulate inflammation (33). However, the issue of whether UTs serve as endogenous metabolite-derived danger signals to activate DAMPs receptors including TLRs and NLRs/inflammasome/caspase-1 remains unknown. To demonstrate a proof of principle that classical DAMP receptors play a critical role in accelerating CKD-promoted vascular inflammation, we recently reported that NLR-inflammasome caspase-1 pathway plays an essential role in sensing CKD-derived DAMPs, and in significantly promoting neointimal hyperplasia formation in carotid artery in 5/6 nephrectomy-induced CKD mouse model (6).

In this study, we collected 116 experimentally identified UTs and examined two novel hypotheses that: *first*, UTs can serve as conditional pro-inflammatory DAMPs, or anti-inflammatory HAMPs, and modulate inflammation; and *second*, in addition to passive accumulation due to decreased glomeruli filtration in CKD, elevation of UTs can be partially induced by classical DAMP receptors such as TLRs, NLR-inflammasome-activated caspase-1, and other pro-inflammatory cytokines as well as be inhibited by $CD4^+Foxp3^+$ regulatory T cells (Tregs). Using a

novel database mining approach, our results have demonstrated for the first time that UTs are selectively increased, and serve as DAMPs and HAMPs to modulate inflammation (30,34); that UT genes including protein carried UT receptors and UT synthases can be upregulated in CKD and CAD presumably via caspase-1/inflammatory cytokine pathways; and that elevation of UTs does not result from purely passive accumulation. The findings have significantly improved our understanding of the molecular mechanisms underlying the roles of UTs in accelerating vascular inflammation and UT generation, which provide novel insights for the future development of novel therapeutics for CKD- and CKD-promoted cardiovascular disease and other diseases.

3. MATERIALS AND METHODS

3.1. Uremic toxins

We analyzed 116 experimentally verified UTs that were identified in recently published reports and review (35–37). The experimental method used in the identification of those UTs was mass spectrometry.

3.2. Expression profiles of uremic toxins and related enzymes and receptors in disease model

Gene expression profiles of the identified UTs were analyzed in 13 microarray datasets extracted from NIH-GEO database (http://www.ncbi.nlm.nih.gov/gds/) (Figure 1). The information regarding metabolite synthesis pathway enzymes was extracted from the Human Metabolome Database (http://www.hmdb.ca/). The information related to genes encoding protein/peptide-based UTs, enzymes, and receptors was obtained from the NCBI-Gene database (http://www.ncbi.nlm.nih.gov/gene/). The UTs which exist in the exosomes are examined in the ExoCarta database (http://www.exocarta.org/) . The information of the UTs can be identified in NIH-NCBI-PubChem Database (https://pubchem.ncbi.nlm.nih.gov/). Specific samples were chosen as disease or treatment groups and parallel control. The number of samples was always greater than 3, except for the pooled samples. We selected the genes with significant expression changes (p<0.0.5) in the microarray dataset and examined the fold change of the genes of our interest. The genes with more than 1-fold expression change were defined as the upregulated genes while genes with their expression changes less than 1-fold were defined as downregulated genes.

3.3. Ingenuity pathway analysis

In order to categorize clinical functions and molecular and cellular functions related to the identified genes in our microarray analysis, the Ingenuity Pathway Analysis (IPA, Ingenuity Systems, www.ingenuity.com) was used. The differentially expressed genes were identified and uploaded into IPA for analysis. The Core pathways analysis was used to identify molecular and cellular pathways.

4. RESULTS

4.1. Uremic toxins represent 1/80th of human serum small-molecule metabolome

To identify the molecular mechanisms of how CKD accelerates vascular inflammation, we hypothesized that CKD selectively accumulates a specific group of endogenous metabolites as UTs (Figure 2). We focused on analyzing the experimentally identified UTs. As shown in Table 1, 116 UTs have been identified (35–37), including four categories: 1) 53 small molecules (<500 Daltons); 2) 30 protein-bound molecules; *3) 3*9 middle-sized molecules, including protein/peptide-based (>500 Daltons); and *4)* 15 microbe-generated toxins (38). Among 53 small-molecule toxins, only one receptor for inosine has been identified. Of note, the Human Metabolome Database identification numbers (IDs) for three small-molecule toxins were not found in the database; and the NIH-NCBI-PubChem Database IDs for five small-molecule toxins were not found in the database, suggesting these toxins are newly identified. In addition, 12 out of 30 protein-bound molecule toxins were found to have their own intrinsic receptors. Moreover, 20 out of 35 protein/peptide-based toxins had their own receptors, including several cytokines such as interleukin-18 (IL-18), IL-6, IL-1β, leptin, tumor necrosis factor-α (TNF-α). One of the microbe-generated UTs, pentosidine, can bind to the receptor for advanced glycation end products (RAGE) (39). As shown in Figure 3, those toxins, whose intrinsic receptors have not been identified, may also use classical DAMP receptors and nuclear receptors (for lipophilic toxins) to initiate inflammation-regulatory functions (29, 40–42). Furthermore, as shown in Table 2, our analysis on an exosome database (ExoCarta database; http://www.exocarta.org/) found that 6 out of 34 protein/peptide-based toxins have been found in exosomes in the plasma, suggesting that those toxins can promote/modulate the target cells for inflammation via exosome uptake mechanism with and without binding to their own receptors (43).

We first argued that if the generation of UTs results from accumulation of endogenous metabolites due to decreased glomeruli filtration in CKD, the compositions of UTs should be proportionally at least similar to, if not the same as, the human plasma metabolome. To our surprise, the most comprehensive Human Metabolome Database (HMDB) (version 3.6.) contains 41,993 metabolite entries, including both water-soluble and lipid-soluble

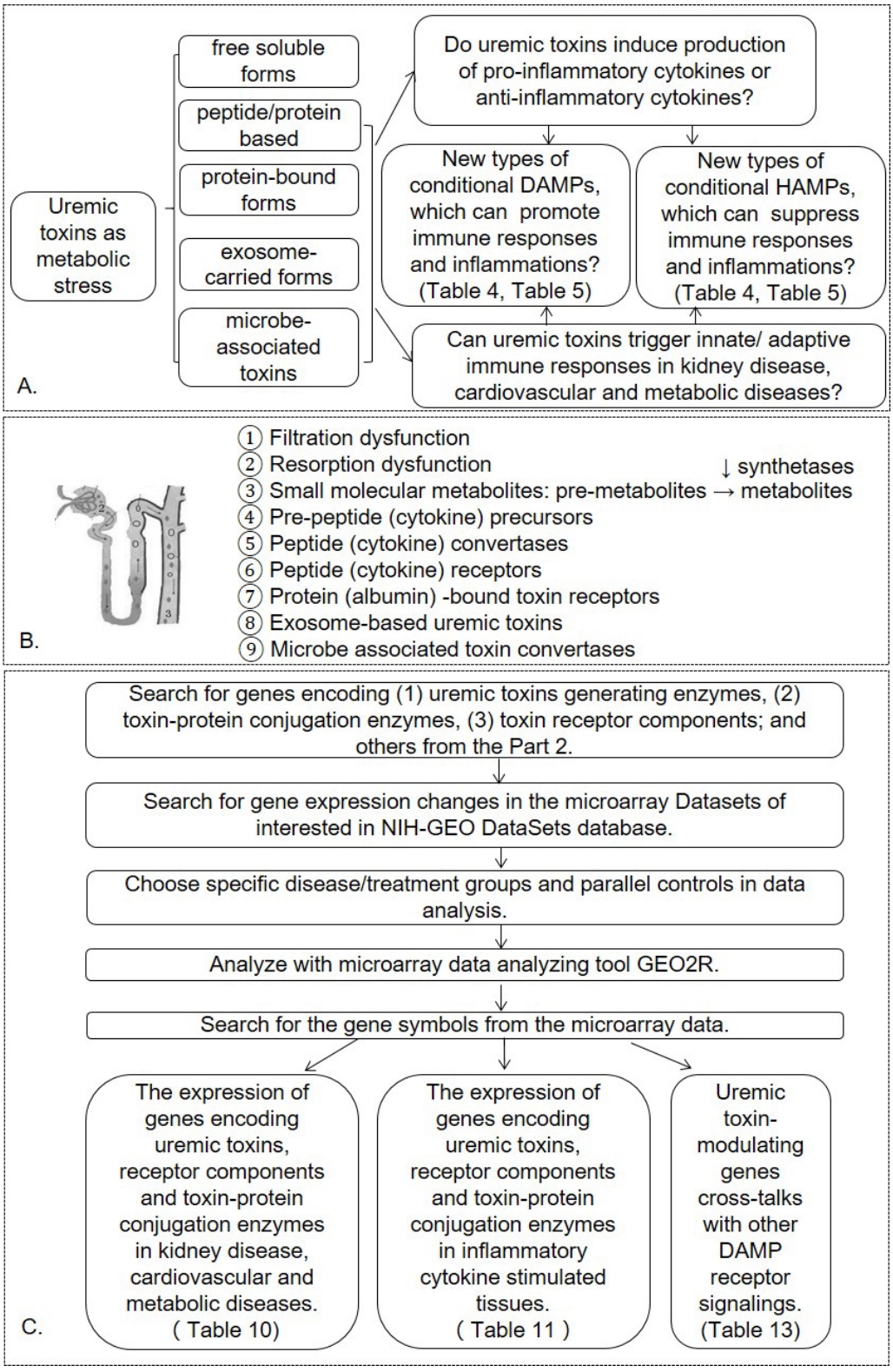

Figure 1. Flow chart of database mining strategy and three parts of data organization. A. We propose a new paradigm that uremic toxins are conditional pro-inflammatory danger-associated molecular pattern molecules (DAMPs) or anti-inflammatory homeostasis-associated molecular pattern molecules (our newly proposed HAMPs). Uremic toxins identified were classified into five groups based on their molecular sizes, molecular structure, molecular carrier and sources. The supporting data for this new paradigm were presented in Tables 3, 4, 5 and Figure 2, respectively. B. Potential pathways for toxin synthase gene upregulation and its signaling components in pathophysiological conditions. The seven pathways from # 3 to #9 were examined in this study. C. Identified uremic toxin genes, their expression data and potential underlying mechanisms were analyzed through data-mining.

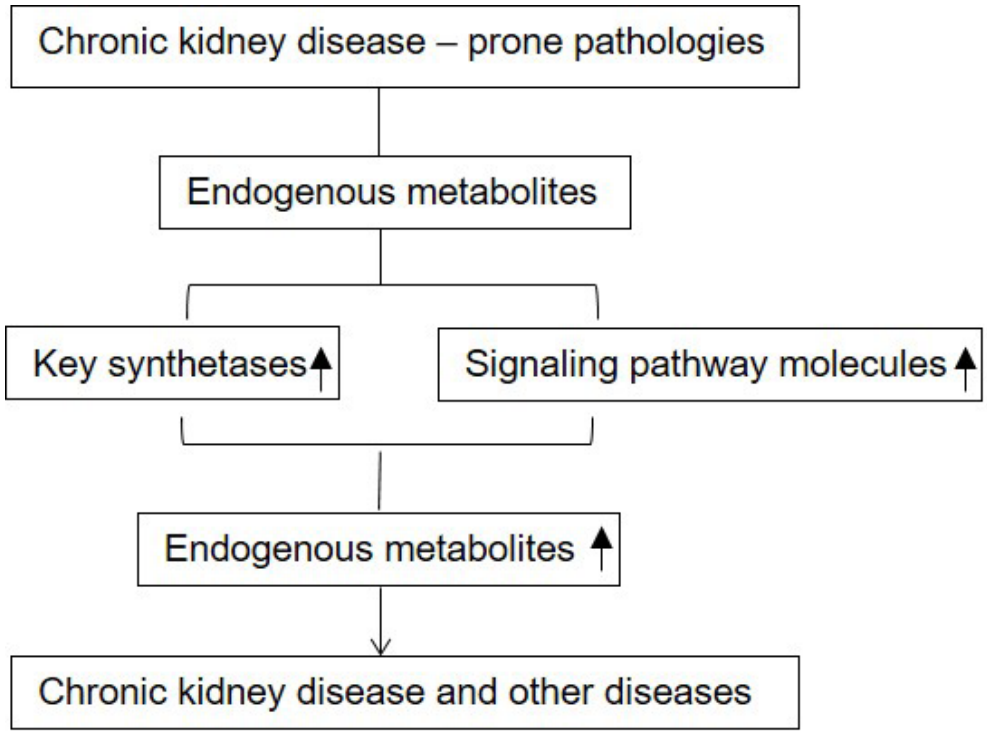

Figure 2. Two novel hypotheses were examined on the expression levels of two sets of genes: First, uremic toxin generating enzymes (Table 6) and second, receptor complex components (Table 7). We examined these two hypotheses to address how chronic kidney disease prone pathologies increase endogenous metabolites.

Table 1. 116 uremic toxins, in four groups, experimentally identified in the plasma of patients with chronic kidney disease

Toxin	Receptors	HMDB	PubChem ID	Gene ID
Group 1. Small molecules (53) (<500Daltons)				
1-Methyladenosine	–	03331	27476	–[1]
1-Methylguanosine	–	01563	96373	–
1-Methylinosine	–	02721	65095	–
8-OH-2′Deoxyguanosine	–	03333	–	–
Asymmetric dimethylarginine (ADMA)	–	01539	123831	–
Arabinitol	–	01851	439255	–
Argininic acid	–	03148	160437	–
Benzyl alcohol	–	03119	244	–
Creatine	–	00064	586	–
Creatinine	–	00562	588	–
Cytidine	–	00089	6175	–
Dimethylglycine	–	00092	673	–
Dimethylguanosine	–	04824	92919	–
Erythritol	–	02994	222285	–
Guanidine	–	01842	3520	–
Guanidinoacetate	–	00128	763	–
Guanidinosuccinate	–	03157	439918	–

Hypoxanthine	–	00157	790	–
Inosine	A2AR, A2R	00195	6021	–
Malondialdehyde	–	06112	10964	–
Mannitol	–	00765	6251	–
Methylguanidine	–	01522	10111	–
Myoinositol	–	00211	892	–
N1-Methyl-2-pyridone-5-carboxamide	–	04193	69698	–
Nitrosodimethylamine	–	31419	6124	–
N2,N2-Dimethylguanosine	–	04824	92919	–
N4-Acetylcytidine	–	05923	107461	–
N6-carbamoyl-Threonyladenosine	–	41623	–	–
N6-Methyladenosine	–	04044	102175	–
Orotic acid	–	00226	967	–
Orotidine	–	00788	92751	–
Oxalate	–	02329	971	–
Phenylacetylglutamine	–	06344	92258	–
Pseudouridine	–	00767	15047	–
Phenylethylamine	–	12275	1001	–
Sorbitol	–	00247	5780	–
Symmetric dimethylarginine (SDMA)	–	03334	169148	–
Thiocyanate	–	01453	9322	–
Taurocyamine	–	03584	68340	–
Threitol	–	04136	169019	–
Thymine	–	00262	1135	–
Trimethylamine	–	00906	1146	–
Uracil	–	00300	1174	–
Urea	–	00294	1176	–
Uric acid	–	00289	1175	–
Uridine	–	00296	6029	–
Xanthine	–	00292	1188	–
Xanthosine	–	00299	64959	–
α-N-Acetylarginine	–	04620	–	–
γ-Guanidinobutyrate	–	03464	500	–
Nitrosomethylamine	–	–	148811	–
α-keto-δ-Guanidinovaleriate	–	–	–	–
β-Guanidinopropionate	–	–	–	–
Group 2. Protein-bound molecules (30)				
Carboxy methyl propyl furanpropionic acid (CMPF)	–	61112	123979	–
Urea	–	00294	1176	–
Homocysteine	–	00742	778	–
Hydroquinone	–	02434	785	–
Indole-3-acetate	–	00197	802	–
Indoxyl sulfate	–	00682	10258	–
Interleukin-18	Il-18R	–	–	3606
Interleukin-6β	IL-6R	–	–	3569

Interleukin-1β	IL-1R	–	–	3553
Leptin	LEPR/OBR	–	90470904	3952
Melatonin	MT1 MT2, RZR/ROR	01389	896	–
Methylglyoxal	–	01167	880	–
p-Creso	–	01858	2879	–
Pentosidine	RAGE	03933	119593	–
Phenol	–	00228	996	–
Phenylacetic acid	–	00209	999	–
Putrescine	–	01414	1045	–
Quinolinic acid	–	00232	–	–
Retinol binding protein	–	–	–	5950
Spermidine	–	01257	1102	–
Spermine	–	01256	1103	–
Tumor necrosis factor-α	TNFR	–	–	7124
2-Methoxyresorcinol	–	–	121805	–
3-Deoxyglucosone	–	–	114839	–
Fructoselysine	–	–	49859675	–
Glyoxal	–	–	7860	–
Kinurenine	AHR	–	846	–
Kinurenic acid	GPR35	–	3845	–
Nε-Carboxymethyllysine	–	–	123800	–
p-OHhippurate	–	–	–	–
Group 3. Mid-size molecules (4) (>500Daltons)				
Hyaluronic acid	GP85/CD44	02061	24728612	–
Octopamine	Octβ2R	04825	4581	–
Dinucleoside polyphosphates	–	–	–	–
Uridineadenosine tetraphosphate (Up4Ab)	–	–	–	–
Protein/peptide based (35) (>500 Daltons)				
Substance P	GPCR, NK-1R, NK-2R, NK-3R	01897	36511	–
Adrenomedullin	CRLR	–	56841671	133
Calcitonin-gene related peptide (CGRP)	CALCRL, RAMP1	–	56841902	796, 797
Ghrelin	GHSR1a	–	44576256	51738
Guanilin	–	–	90488722	2980
Leptin	LEPR/OBR	–	90470904	3952
Orexin A	OX1R, OX2R	–	56842143	3060
Parathyroid hormone	PTH1R	–	16129682	5741
Uroguanylin	GC-C, GC-D	–	5488765	2981
Vasoactive intestinal peptide	VPAC1, VPAC2	–	16129679	7432
Adiponectin	AdipoR1, AdipoR2	–	–	9370
Atrial natriuretic peptide	NPR1, NPR2, NPR3	–	–	4878
Basic fibroblast growth factor	bFGF-R1, bFGF-R2	–	–	2247
Cholecystokinin	CCK1R, CCK2R	–	–	885
Clara cell protein	–	–	–	7356
Complement factor D	–	–	–	1675

Cystatin C	–	–	–	1471
Endothelin	ETA, ETB1, ETB2, ETC	–	–	1906
Hepcidin	ferroportin	–	–	744861
Interleukin-18	Il-18R	–	–	3606
Interleukin-6β	IL-6R	–	–	3569
Interleukin-1β	IL-1R	–	–	3553
Motiline	–	–	–	4295
Neuropeptide Y	NPY1R, NPY2R, NPY3R, NPY4R, NPY5R, NPY6R,	–	–	4852
Retinol binding protein	–	–	–	5950
Tumor necrosis factor-α	TNFR	–	–	7124
β2-Microglobulin	–	–	–	567
κ-Ig Light chain	–	–	–	3514
λ-Ig Light chain	–	–	–	3535
Des-acylghrelin (DAG)	–	–	90488789	–
Methionine-enkephalin	–	–	6427062	–
δ-Sleep-inducing peptide	–	–	3623358	–
degranulation-inhibiting protein-I, (DIP I)	–	–	–	–
β-Endorphin	–	–	–	–
β-Lipotropin	–	–	–	–
Group 4. Microbe-associated toxins (15)[2] (<500Daltons)				
Creatinine	–	00562	588	–
Guanidine	–	01842	3520	–
Urea	–	00294	1176	–
Indole-3-acetate	–	00197	802	–
Indoxyl sulfate	–	00682	10258	–
p-Creso	–	01858	2879	–
Phenol	–	00228	996	–
Phenylacetic acid	–	00209	999	–
Phenylacetylglutamine	–	06344	92258	–
Pentosidine[3]	RAGE	03933	119593	–
Putrescine[3]	–	01414	1045	–
Spermidine[3]	–	01257	1102	–
Spermine[3]	–	01256	1103	–
Trimethylamine	–	00906	1146	–
Uric acid[3]	–	00289	1175	–

[1] The IDs of the uremic toxins are not available in the databases. [2] they are normal metabolites of tryptophan, but the increased excretion in uremia is from bacterial degradation. Reference PubMed ID: 19234110, PMID 19946322, PMID 25198138. [3] the five uremic toxins are generated both in body tissue and in bacteria

metabolites as well as metabolites, that would be regarded as either abundant (>1μM) or relatively rare (<1nM) (http://www.hmdb.ca/). Obviously, not every metabolite appears in the human serum or plasma. Indeed, a recent report showed that 4,229 metabolites, roughly 10% of the total metabolites, have been identified in human serum metabolome (http://www.serummetabolome.ca)[44]. In addition, the Serum Metabolome database collected 4,651 small-molecule metabolites found in human serum (http://www.serummetabolome.ca/). Although these datasets did not result from the same studies, our analysis results, tentatively taken together, showed that roughly 1/80th, a very small fraction, of total human serum small-molecule metabolome were selectively accumulated in patients with CKD. If similar efficiencies were presumably achieved in identifying metabolites in human plasma and UTs with

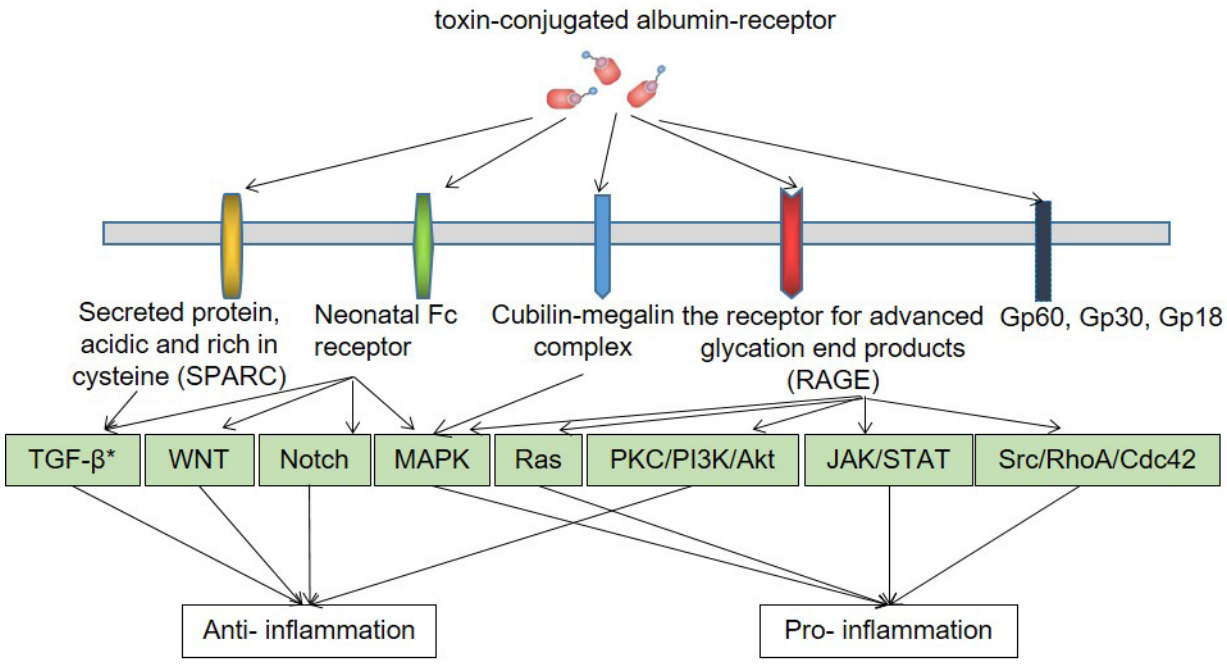

Figure 3. Protein-bound uremic toxins could take use of five potential toxin-conjugated albumin-receptor pathways to either promote or suppress inflammation. There are two features for these five pathways: first, each receptor may signal several pathways; and second, downstream pathways connecting to each receptor can be shared. * TGF-β: Transforming growth factor beta; WNT: Wnt signaling pathways; Notch: Notch Signaling Pathway; MAPK: Mitogen-activated protein kinases; Ras: The Ras family; PKC/PI3K/Akt: Protein kinase C, phosphoinositide 3-kinases, protein kinase B; JAK/STAT: Janus kinase/signal transducer and activation of transcription; Src/RhoA/Cdc42: Proto-oncogene tyrosine-protein kinase Src, Ras homolog gene family, member A, Cell division control protein 42 homolog (Ref number 14517321, 25674083, 26055641, 25974754, 26925240).

Table 2. 6 uremic toxins have been identified in exosomes in plasma

Uremic Toxin	Gene symbol	Gene ID
β2-Microglobulin	B2M	567
Complement factor D	CFD	1675
Cystatin C	CST3	1471
κ-Ig Light chain	IGK	50820
λ-Ig Light chain	IGL	3535
Retinol binding protein	RBP4	5950

the current technologies, these analyses suggest that *first*, highly specific metabolites are highly selectively increased in the plasma of patients with CKD; and *second*, the high specificity of UTs accumulated in patients with CKD may not fully result from passive accumulation due to kidney malfunction in filtrating metabolites into urine. Instead, active mechanisms in synthesizing, processing, or converting these UTs may be the key regulating events for elevation of those toxins.

4.2. Classification of uremic toxins as DAMPs or HAMPs

We recently proposed a new paradigm that pathologically elevated endogenous metabolites can be categorized into either conditional pro-inflammatory danger-associated molecular patterns (DAMPs) or anti-inflammatory homeostasis-associated molecular patterns (HAMPs) based on the roles of these metabolites in regulating inflammation (33). To determine whether UTs are conditional DAMPs or HAMPs, we searched the Human Metabolome Database for the concentrations of toxins in physiological and pathological conditions. As shown in Table 3, among 69 UTs that can be found in the Human Metabolome Database, 24 (35%) UTs are unique to CKD, the remaining 45 (65%) UTs are shared with other diseases/CVD risk factors, including various cancers, smoking, hypertension, Alzheimer's disease, cirrhosis, Canavan disease, etc. Among these 24 CKD UTs, 11 toxins had no reports on the physiological and pathological concentrations for comparison in the Human Metabolome Database. Seven CKD UTs were

significantly increased more than 5-fold, including 3-Carboxy-4-methyl-5-propyl-2-furan propionate (CMPF, causing proximal tubular cell damage) (8-fold) (45); dimethylguanosine (altered RNA metabolism in patients with CKD) (14.2.-fold) (46); methylguanidine (26-fold); N2,N2-dimethylguanosine (14.2.-fold); p-Cresol (causing growth retardation) (>10-fold) (47); phenol (9.1.5-fold); and taurocyamine (inducing convulsive seizures; https://www.wikigenes.org/e/chem/e/68340.html) (7.8.-fold). The concentrations of two CKD toxins were actually decreased, including spermidine and spermine, which may cause the arrest in protein translation and cell growth (48). Of note, the concentration changes in other diseases may either be increased or decreased in comparison to those of physiological conditions. These results suggest that these UTs that are changed in other diseases with opposite directions may contribute differently to the pathogenesis of those diseases. Among 24 CKD-specific UTs, the pathological concentrations of 10 toxins are available for the analysis, which are all increased in the pathological conditions, suggesting that these UTs are the conditional DAMPs.

Next, in order to verify UTs are conditional DAMPs or HAMPs, we examined our new hypothesis that UTs regulate inflammation, by either inducing or suppressing the expression of pro-inflammatory cytokines. To test this hypothesis, we searched for the experimental evidence that UTs can induce the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-18, IL-6, monocyte chemoattractant protein-1 (MCP-1), adhesion molecules, nuclear factor-kB (NF-κB) signaling molecules or mitogen-activated protein kinases (MAPK) signaling molecules, etc. As shown in Table 4, among 92 free UTs, we found experimental reports showing that 32 UTs regulate inflammation in various cell types, with 20 promoting inflammation (as DAMPs, 62.5%) and 12 inhibiting inflammation (as HAMPs, 37.5.%). Moreover, as shown in Table 5, among 30 protein-bound UTs, we found via searching experimental reports that 19 protein-bound UTs regulate inflammation (63.3%) in various cell types, with 14 promoting inflammation as DAMPs (73.7%) and 5 inhibiting inflammation as HAMPs (26.3.%). These results suggest that regardless of whether UTs are bound to carrier proteins or not, UTs promote, more than inhibit, inflammation; and that more protein-bound UTs (73.7% versus 62.5%) than free UTs promote inflammation.

Finally, our Ingenuity Pathway Analysis (IPA) results of protein/peptide-based UTs indicated that the top ten pathways for those protein/peptide-based UTs (Figure 4) include: 1) communication between innate and adaptive immune cells; 2) hepatic fibrosis/hepatic stellate cell activation; 3) dendritic cell maturation; 4) role of hypercytokinemia/hyperchemokinemia in the pathogenesis of inflammation (influenza); *5)* graft-versus-host disease signaling; *6)* liver X nuclear receptor (LXR/RXR) activation (important regulators of cholesterol, fatty acid, and glucose homeostasis); *7)* atherosclerosis signaling; *8)* role of cytokines in mediating communications between immune cells; *9)* differential regulation of cytokine production in macrophages and T helper cells by interleukin-17A (IL-17A) and IL-17F; and *10)* IL-10 signaling. Once again, the IPA results strengthen our arguments that protein/peptide-based UTs have more pro-inflammatory than anti-inflammatory functions.

4.3. Uremic toxins facilitate CAD in patients with CKD

Our above-described results indicate that roughly 1/80th, a very small fraction, of total human serum small-molecule metabolome was selectively accumulated in patients with CKD. The results suggest that the high specificity of UTs accumulated in patients with CKD may not result from passive accumulation due to kidney malfunction in filtrating metabolites into urine. We hypothesized that active mechanisms in synthesizing, processing, or converting these UTs may be the key regulating events for elevation of those toxins. To test this hypothesis, we first searched the toxin-generating enzymes. Among 69 UTs that can be found in the Human Metabolome Database, the 67 generating enzymes for 33 toxins can be found as shown in Table 6. In addition, for 30 protein-bound UTs that may mainly bind to serum albumin, albumin-bound UTs may initiate inflammation-regulatory signaling via binding to five potential receptor complexes and their signaling components, including glycoproteins (Gp60, Gp30 and Gp18) (49–51); secreted protein acidic and rich in cysteine (SPARC, 8 genes) (50); neonatal Fc receptor (FcRn, 15 genes), cubilin-megalin (9 genes), and receptor for advanced glycation end products (RAGE, 23 genes), totaling 60 genes, as shown in Table 7 (50,52). Of note, some signaling components are shared among the receptor pathways. Moreover, as shown in Table 8, among 34 protein/peptide-based UTs, the convertases for generating 14 out of 34 UTs have been identified. Furthermore, since exosomes are identified as a potential key carrier for CKD-driven cardiovascular disease, we found that 28 out of 169 genes that have been identified in exosomes in the ExoCarta exosome database, which indicate these UTs can use exosome uptake mechanisms to initiate inflammation-modulating pathways as shown in Table 9 (53). Taken together, we collected 169 genes that generate UTs (Table 6) and mediate UT signaling (Tables 7, 8 and 9).

To examine our hypothesis that the expression of these 169 genes is partially modulated in various diseases (Figure 5) including CKD, we mined the microarray database in the NIH-GEO Datasets as shown in Table 10. Our

Table 3. The supporting evidence 1 for classifying uremic toxins as conditional DAMPs or HAMPs: The uremic toxins are elevated in the plasma of patients with CKD and other diseases (69)

Toxin	Physiological Concentration	Pathological Concentration	Pathological/ Physiological concentration ratio	Elevation in Other Disease	PMID
Metabolites elevated only in CKD (24)					
Benzyl alcohol	–	–	–	–	–
CMPF	4.6 ± 4.2 μM	36.63 ± 20.81 μM	7.96	–	–
Dimethylglycine	1.8–3.7 μM	3.1–7.2 μM	5.15	–	–
Dimethyl guanosine	0.031 ± 0.004 μM	0.44 ± 0.09 μM	14.19	–	–
Guanidinoacetate	2.8 ± 0.9 μM	2.4 ± 0.7 μM	0.86	–	–
Hydroquinone	–	–	–	–	–
Indoxyl sulfate	14.0 ± 4.2 μM	21.11 ± 12.20 μM	1.51	–	–
Melatonin	0.000063 ± 0.000026 μM	–	<15873	–	–
Methylguanidine	0.0–0.05 μM	3.3 ± 1.3 μM	132	–	–
Nitrosodimethylamine	–	–	–	–	–
N2,N2-Dimethylguanosine	0.031 ± 0.004 μM	0.44 ± 0.09 μM	14.19	–	–
N4-Acetylcytidine	–	–	–	–	–
N6-Methyladenosine	–	–	–	–	–
N6-carbamoyl-Threonyladenosine	–	–	–	–	–
Orotidine	149.0 ± 13.0 μM	–	<0.00	–	–
p-Cresol	–	9.9 ± 5.1 μM	>9.9	–	–
Phenol	6.38 ± 2.13 μM	58.44 ± 39.32 μM	9.16	–	–
Phenylacetylglutamine	3.34 ± 0.31 μM	–	<0.30	–	–
Phenylethylamine	–	–	–	–	–
Spermidine	10.3 ± 3.78 μM	0.069 ± 0.053 μM	0.00	–	–
Spermine	9.97 ± 3.26 μM	0.0092 ± 0.0076 μM	0.00	–	–
Thiocyanate	30.7 ± 28.8 μM	32.02 ± 2.93 μM	1.04	–	–
Taurocyamine	0.33 μM	2.56 μM	7.76	–	–
Xanthosine	5.08 ± 0.30 μM	–	<0.20	–	–
Metabolites also elevated in other diseases (45)					
1-Methyladenosine	0.10 ± 0.03 μM	0.078 ± 0.031 μM	0.07	Cervical cancer	7482520
				Cholangiocarcinoma	7482520
				Colorectal cancer	7482520
				Stomach cancer	7482520
				Hepatocellular carcinoma	7482520
				Leukemia	7482520
				Ovarian cancer	7482520
1-Methylguanosine	0.046 ± 0.019 μM	0.099 ± 0.021 μM	2.15	Perillyl alcohol administration for cancer treatment	15607313
1-Methylinosine	0.0680 ± 0.022 μM	–	–	Thyroid cancer	9129323
8-OH-2′Deoxyguanosine	0.002 ± 0.0008 μM	0.0037 ± 0.00021 μM	1.85	Smoking	18029489

Uremic toxins are danger patterns or homeostasis patterns.

Asymmetric dimethylarginine (ADMA)	0.28–0.42 μM	4.35 ± 0.19 μM	15.54	Autosomal dominant polycystic kidney disease	18215696
				Essential hypertension	10218738
Arabinitol	0.0–5.0 μM	32.0–198.0 μM	46	Alzheimer's disease	8595727
				Ribose-5-phosphate isomerase deficiency	14988808
Argininic acid	0.015–0.44 μM	0.015–0.5 μM	1.14	Cirrhosis	7752905
Creatine	54.8 ± 21.0 μM	33.8 ± 37.7 μM	0.62	Cirrhosis	7752905
				Lung Cancer	22157537
				Rhabdomyolysis	12089184
Creatinine	82.6 ± 26.2 μM	86.9 ± 44.5 μM	1.05	Canavan disease	16139832
				Hyperoxalemia	15353324
				Paraquat poisoning	9625050
Cytidine	0.25±0.19 μM	0.26 ± 0.13 μM	1.04	Canavan disease	16139832
Erythritol	4.10 ± 1.64 μM	–	<0.24	Ribose-5-phosphate isomerase deficiency	14988808
Guanidine	0.06–0.2 μM	3.1 ± 1.1 μM	23.85	Cirrhosis	7752905
Guanidinosuccinate	0.37–1.13 μM	0.11 ± 0.106 μM	0.30	Cirrhosis	7752905
Hippuric acid	16.74 ± 11.16 μM	486.68 ± 344.36 μM	29.07	Lung cancer	18953024
				Paraquat poisoning	9625050
Homocysteine	7.3–16.2 μM	68.80 ± 15.53 μM	5.86	Alzheimer's disease	11959400
				Continuous ambulatory peritoneal dialysis	11380380
				Creutzfeldt-Jakob disease	15711082
				Dementia	17384003
Hyaluronic acid	–	0.04–10.52 μM	>5.28	Biliary atresia	17875085
				Epilepsy	12121313
				Hepatitis	17875085
Hypoxanthine	11.02 ± 3.67 μM	5.7 ± 0.4 μM	0.52	Canavan disease	16139832
				Degenerative disc disease	6656991
				Hydrocephalus	2611770
				Lesch-Nyhan syndrome	3148065
				Lung Cancer	18953024
Indole-3-acetate	2.85 ± 1.71 μM	13.70 ± 12.56 μM	4.81	Appendicitis	11462886
				Irritable bowel syndrome	9505884
Inosine	0.20 ± 0.07 μM	0.68 ± 0.47 μM	3.4	Canavan disease	16139832
				Coronary artery disease	10499868
				Critical illnesses	9663253
				Degenerative disc disease	6656991
				Purine nucleoside phosphorylase deficiency	8595732
Malondialdehyde	0.69 ± 0.13 μM	5.40 ± 0.30 μM	7.83	Parkinson's disease	17145675
				Smoking	18029489

Uremic toxins are danger patterns or homeostasis patterns.

Mannitol	34.0 ± 18.0 μM	1.14–2.12 μM	0.05	AIDS	8748311
				Alzheimer's disease	8595727
				Cytochrome C oxidase deficiency	7710082
				Lung Cancer	18953024
				Ribose-5-phosphate isomerase deficiency	14988808
Methylglyoxal	0.44–0.74 μM	2.4–3.6 μM	5.08	Diabetes mellitus type 2	18760976
Myoinositol	24.0 ± 7.8 μM	23.0–24.0 μM	0.98	Alzheimer's disease	8595727
				Cachexia	18953024
				Ribose-5-phosphate isomerase deficiency	1498808
N1-Methyl-2-pyridone-5-carboxamide	9.00 ± 4.47 μM	51.27 ± 23.66 μM	5.70	Pellagra	17709435
Octopamine	0.0026 ± 0.0014 μM	0.0026 ± 0.0024 μM	1	Cirrhosis	3137238
				Hypertension	8255371
Orotic acid	0.89 ± 0.63 μM	0.94 ± 0.78 μM	1.06	Canavan disease	16139832
Oxalate	6.43 ± 1.06 μM	47.2 ± 22.9 μM	7.34	Hemodialysis	15353324
Pentosidine	0.14 ± 0.05 μM	1.53 ± 0.79 μM	10.93	Alzheimer's disease	12498967
				Multi-infarct dementia	12498967
Phenylacetic acid	47.24 ± 5.866 μM	3490.0 ± 330.0 μM	73.88	Phenylketonuria	2091926
Pseudouridine	3.18 ± 0.99 μM	16.70 ± 3.72 μM	5.25	Canavan disease	16139832
Putrescine	0.214 ± 0.08 μM	0.11 ± 0.09 μM	0.51	Pancreatic cancer	2315288
Quinolinic acid	0.47 ± 0.047 μM	–	<2.13	AIDS	9657528
				Anemia	12964115
				TraμMatic brain injury	15206793
Sorbitol	1.09 ± 0.37 μM	–	<0.92	Alzheimer's disease	8595727
Substance P	3.6e-6 ± 1.8e-6 μM	4.9e-6 ± 2.7e-6 μM	2.03	Migraine	17123735
Symmetric dimethylarginine (SDMA)	0.368–0.552μM	2.08 ± 0.11 μM	5.65	Autosomal dominant polycystic kidney disease	18215696
Trimethylamine	0.42 ± 0.12 μM	1.38 ± 0.48 μM	3.29	Trimethylaminuria	9246418
Threitol	0.0–5.0μM	5.0–8.0μM	3	Ribose-5-phosphate isomerase deficiency	14988808
Thymine	–	1390.0 ± 150.0 μM	>1390.0	Beta-ureidopropionase deficiency	15385443
				Thymidine treatment	6736109
Uracil	2.10 ± 1.02 μM	2.25 ± 0.98 μM	1.07	Canavan disease	16139832
				Hypertension	9816152
Urea	6074.6 ± 2154.2 μM	3500.0 ± 1500.0 μM	0.58	Cirrhosis	7752905
				Meningitis	15627241
				Tuberculous meningitis	15627241

Uremic toxins are danger patterns or homeostasis patterns.

Uric acid	377.6 ± 82.6 μM	400.0 ± 103.2 μM	1.06	Adenylosuccinate lyase deficiency	15571235
				Bacterial meningitis	17942520
				Cachexia	11320368
				Canavan disease	16139832
				Degenerative disc disease	6656991
				Diabetes mellitus type 2	11887176
				Impaired glucose tolerance	11887176
				Lesch-Nyhan syndrome	15804753
				Meningitis	11805243
				Multiple sclerosis	11985629
Uridine	2.90–3.30 μM	7.7 ± 0.9 μM	2.66	Canavan disease	16139832
				Degenerative disc disease	6656991
				Lesch-Nyhan syndrome	3148065
Xanthine	1.27 ± 0.78 μM	2.2 ± 0.3 μM	1.73	Canavan disease	16139832
				Degenerative disc disease	6656991
				Hydrocephalus	2611770
				Lesch-Nyhan syndrome	3148065
α-N-Acetylarginine	1.25 ± 0.28 μM	–	<0.8	Hyperargininemia	3433275
γ-Guanidinobutyrate	0.013–0.055 μM	0.013–0.09 μM	1.51	Cirrhosis	7752905
Mannitol	34.0 ± 18.0 μM	1.14–2.12 μM	0.05	AIDS	8748311
				Alzheimer's disease	8595727
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Orotic acid	0.89 ± 0.63 μM	0.94 ± 0.78 μM	1.06	Canavan disease	16139832
Oxalate	6.43 ± 1.06 μM	47.2 ± 22.9 μM	7.34	Hemodialysis	15353324
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Quinolinic acid	0.47 ± 0.047 μM	–	<2.13	AIDS	9657528
				Anemia	12964115
				Traμmatic brain injury	15206793

Sorbitol	1.09 ± 0.37 μM	–	<0.92	Alzheimer's disease	8595727
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Threitol	0.0–5.0μM	5.0–8.0μM	3	Ribose-5-phosphate isomerase deficiency	14988808
Thymine	–	1390.0 ± 150.0 μM	>1390.0	Beta-ureidopropionase deficiency	15385443
				Thymidine treatment	6736109
Uracil	2.10 ± 1.02 μM	2.25 ± 0.98 μM	1.07	Canavan disease	16139832
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Urea	6074.6 ± 2154.2 μM	3500.0 ± 1500.0 μM	0.58	Cirrhosis	7752905
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				Lesch-Nyhan syndrome	3148065
α-N-Acetylarginine	1.25 ± 0.28 μM	–	<0.8	Hyperargininemia	3433275
γ-Guanidinobutyrate	0.013–0.055 μM	0.013–0.09 μM	1.51	Cirrhosis	7752905

analysis of microarray experimental data indicated that 14 UT genes were upregulated; and another 14 UT genes were downregulated in the CKD kidney tubules. In addition, we found that 7 UT genes were upregulated; and another 9 UT genes were downregulated in the adipose tissues of patients with coronary artery disease (CAD). The striking similarities have been noted in upregulated pro-inflammatory pathways, including pro-inflammatory caspase-1 and caspase-1 substrate IL-18 in both CKD kidney tubules and CAD adipose tissue, suggesting that the same upregulated pro-inflammatory pathways underlie the pathogenesis of CKD and CAD. Moreover, we observed that 3 UT genes were upregulated and another 16 UT genes were slightly downregulated in the peripheral blood cells of patients with metabolic syndrome. Furthermore, we identified that 4 UT genes were upregulated and another 12 UT genes were slightly downregulated in the liver of patients with type 2

Table 4. The supporting evidence 2 for classifying uremic toxins as DAMPs or HAMPs: Free uremic toxins either promote (DAMPs) or inhibit inflammation (HAMPs)

Metabolite	Concentration	Cell type/tissue	Induced cytokines/ signaling	Suppressed cytokines/ signaling	PMID
Molecules promoting inflammation (20)					
Asymmetric dimethylarginine (ADMA)	3 μM 10 μM 30 μM	Human monocytoid cells	NF–κB	–	18295546
Basic fibroblast growth factor	50μl of 50μg	Inbred male Lewis rats	ICAM-1, P-selectin, E-selectin	–	16507899
Clara cell protein	10 M	Human bronchiolar epithelium	–	IL-2, IFN-γ	7865218
Cystatin C	–	Hypertension patient blood	TNF-α, IL-6, CRP	–	20809110
Endothelin	–	–	NF-κB, MAPKs, TNF-α, IL-1,	–	25288367
Ghrelin	1 ng/ml	HUVEC	–	IL-1α, IL-1β, IL-6, TNF-α, IL-8, MCP-1	21565248
Guanidinoacetate	1.88 μM	Human blood	TNF-α	–	18048424
Guanidinosuccinate	8.27 μM	Human blood	–	Neutrophil superoxide production, Natural killer cell response to interleukin-2	18048424
Interleukin-18	–	–		–	16470011
Interleukin-1β	–	–	L-1β	–	16470011
Interleukin-6β	–	–	IL-6β	–	16470011
Malondialdehyde	50 μmol/L	Human peripheral blood lymphocytes (PBLCs)	IL-25, IL-6, IL-8, ICAM-1, PKC, p38MAPK, NF-κB,	–	22956781
Methylguanidine	1.91 μM	Human blood	TNF-α	–	18048424, 17324147
Neuropeptide Y	0.02 g/L	Y1-deficient mice	IL-12,TNF-α, NO, IL-4, adenylate cyclase-cAMP, NF-κB, COX-2, MAPK, PKA, phospholipase C, PKC, phosphatidyl inositol-3-kinase	IFN-γ	23538492
Parathyroid hormone	46.3pg/mL	Human blood	CRP	–	24782595
Retinol binding protein	–	HRCEC and HUVEC	VCAM-1, ICAM-1, E-selectin, MCP-1, IL-6	–	23071093
Symmetric dimethylarginine (SDMA)	1.5 μM, 3.0 μM, 6.0 μM, 12.0 μM, 36.0 μM	Human blood	Monocytic ROS production	–	19059932
Substance P	2.0 μM	Human mast cells	IL-8, TNF, VEGF	–	1701206
Tumor necrosis factor-α	–	–	TNF-α	–	23095282
Uric acid	–	–	VSMC, proliferation, MAPK, NF- κ B IL-1β	–	15660333, 21234729
Molecules inhibiting inflammation (12)					
8-OH-2′Deoxyguanosine	60 mg/kg	Bal b/c mice	–	TNF-α, IL-6, IL-18, IL-12p70, NF-κB, c-Jun	18037125
Adiponectin	–	Human aortic endothelial cells	IL-10	TNF-α, VCAM-1, E-selectin, ICAM-1, IL-8 ,NF-κB, MEK/ ERK signaling pathway, cAMP-PKA	17343838
Adrenomedullin	20.0 ng/kg	Male CD mice	IL-10	iNOS, NF-κB, TNF-α, IL-1β	22685374
Creatine	0.5 mM, 5 mM	Human pulmonary endothelial cells	–	ICAM-1 expression, E-selectin expression	12812994

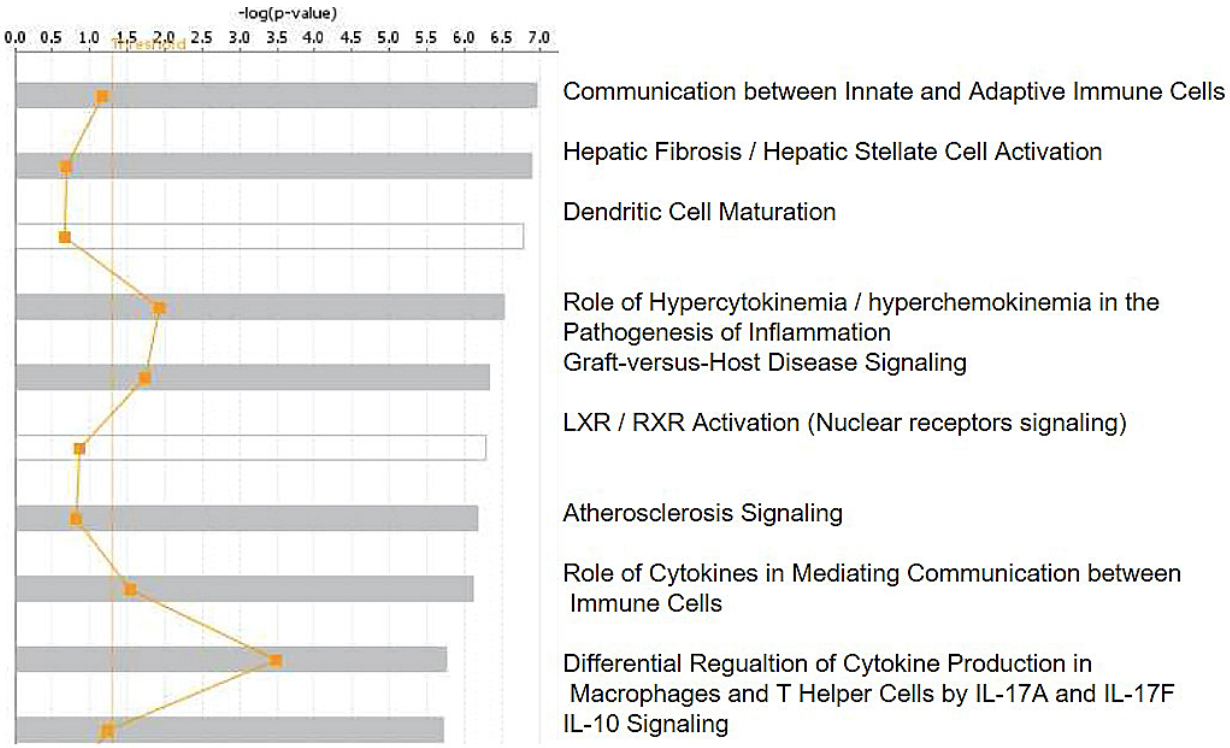
Cholecystokinin	–	kidney tissues of mice	–	CD68, ICAM-1, TGF-β, TNF-α, NF-κB	22357963
Hyaluronic acid	0.1mg/ml, 1mg/ml, 2mg/ml, 3mg/ml, 5mg/ml	Human rheumatoid arthritis synovial tissues	–	IL-1-induced MMP-1 production, TNF-α, MAPK, NF- κ B, p38	20360891
Inosine	100mg/Kg	Cecal ligation and puncture mice	–	TNF-α, IL-1β, IL-6, macrophage inflammatory protein-2	23355189
Leptin	–	ob/ob mice	IL-4	TNF-α, IL-6, IL-1β, JAK-STAT, PI3K, ERK 1/2.	16879738
Orexin A	–	–	–	IL-6 and TNF-α	25884812
Thiocyanate	400.0 μM	Airway-targeted ENaC–overexpressing mice murine macrophage-like cells	–	KC,IL-1b, TNF-α,	25490247
Uridine	80 μl, 24 μg/ml	Male C57BL/6 mice bronchoalveolar lavage (BAL) fluid; human neutrophils	–	IL-6, IL-8, TGF-β, ROS	26369416
Vasoactive intestinal peptide (VIP)	–	Human lymph node immune cells	Foxp3, TGF-β	IL-6, TNF-α, IL-12, NO, TLR-2/TLR-4 expression, CXCL1 production	23538492

Table 5. Protein-bound uremic toxins promote or inhibit inflammation

Metabolite	Concentration	Cell type/tissue	Induced cytokines/ signaling	Suppressed cytokines/ signaling	PMID
Molecules promoting inflammation (14)					
Glyoxal	μM	HUVEC	COX-2, ERK		18343213
Homocysteine	100 μM5–300μ M	HEACR at VSMCs	NF-κB, Proliferation of VSMCs	–	17822365
Hydroquinone	10 μM, 100 μM,	Wistar rat	VCAM-1, ICAM-1, IL-1β, TNF- α,, NF-κB	–	21645265
Indoxyl sulfate	125 μg/mL, 250 μg/mL	HUVECR at VSMC	MAPK	Endothelial proliferation, wound repair	14717914, 18941374
Interleukin-18	–	–		–	16470011
Interleukin-1β	–	–	L-1β	–	16470011
Interleukin-6β	–	–	IL-6β	–	16470011
Methylglyoxal	56–420 μM	HUVEC	JNK, p38 MAPK	–	18842828
p-Cresol	10μ g/mL, 25 μg/mL, 50 μg/mL	HUVEC	–	endothelial proliferation, wound repair	14717914
Pentosidine	229 pmol/ml	Human blood	NF-κB, IL-6	–	15580352
Phenol	50 μM	Human Caco-2 cells	IL-6, IL-8, MCP-1	–	20816778
Phenylacetic acid	5.0 mM	Rat VSMC	iNOS	–	
Retinol binding protein	–	HRCEC and HUVEC	VCAM-1, ICAM-1, E-selectin, MCP-1, IL-6	–	23071093
Tumor necrosis factor-α	–	–	TNF-α	–	23095282
Molecules inhibiting inflammation (5)					
Leptin	–	ob/ob mice	IL-4	TNF-α, IL-6, IL-1β, JAK-STAT, PI3K, ERK 1/2.	16879738
Melatonin	1 mg/kg/day	Male -accelerated mice	IL-10	TNF-α, IL-1β	20817086
putrescine	12.5 mg/Kg	Wistar strain rats liver	amount of malondialdehyde	Lipid peroxide	20040939
Spermidine	3.5 mg/Kg	Wistar strain rats liver	amount of malondialdehyde	Lipid peroxide	20040939
Spermine	2.5 mg/Kg	Wistar strain rats liver	amount of malondialdehyde	Lipid peroxide	

Uremic toxins are danger patterns or homeostasis patterns.

A.



B.

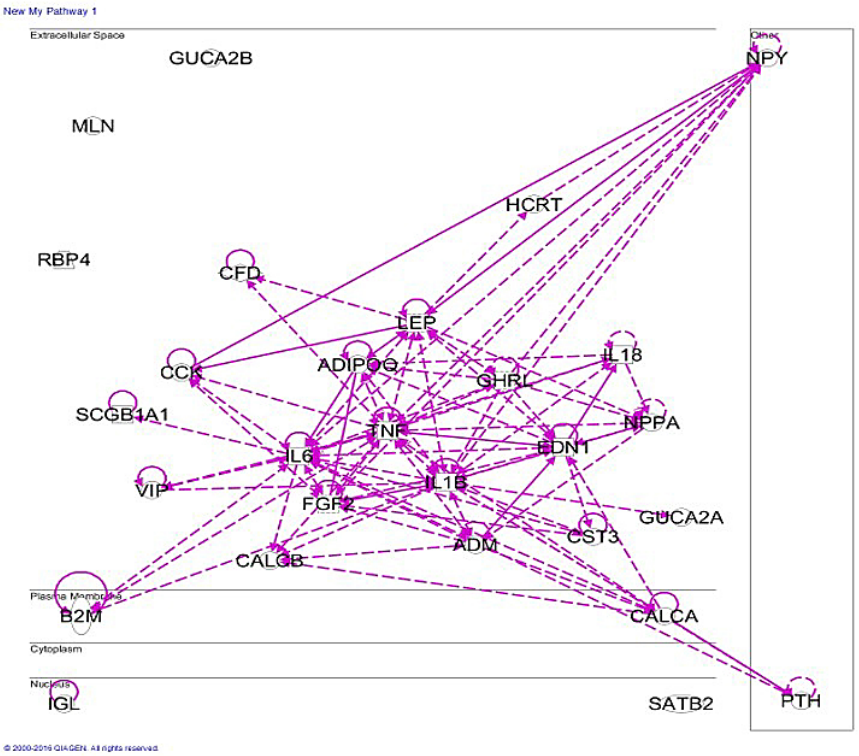


Figure 4. The core analysis with the Ingenuity Pathway Analysis (IPA) suggest that peptide/protein-based uremic toxins play critical roles in promoting immune/inflammatory responses. A. On the left panel, top 10 pathways were identified for peptide/protein-based uremic toxins by The IPA. On the right panel, the relative significance scores were presented for the IPA selection of the top 10 pathways. B. The network shows the pathways of the peptide/protein based uremic toxins were inter-connected.

Table 6. The uremic toxin generating enzymes may be the key regulators for elevation of the toxins in the plasma of patients with chronic kidney disease

Toxin (33)	enzymes	Enzyme Gene Name	NCBI-Gene ID	PMID
Arabinitol	Aldose reductase	AKR1B1	231	25722213
	Aldo-keto reductase family 1 member B10	AKR1B10	57016	25686905
Creatine	Guanidinoacetate N-methyltransferase	GAMT	2593	26202197
Cytidine	5'-nucleotidase	NT5E	4907	25677906
	Cytosolic 5'-nucleotidase 1B	NT5C1B	93034	11690631
	Cytosolic 5'-nucleotidase 1A	NT5C1A	84618	19352542
	5' (3')-deoxyribonucleotidase, cytosolic type	NT5C	30833	15136231
	5' (3')-deoxyribonucleotidase, mitochondrial	NT5M	56953	24506201
	Cytosolic purine 5'-nucleotidase	NT5C2	22978	25857773
	Cytosolic 5'-nucleotidase 3	NT5C3	101125212	–
Dimethylglycine	Betaine–homocysteine S-methyltransferase 1	BHMT	635	25144858
	S-methylmethionine–homocysteine S-methyltransferase	BHMT2	23743	18457970
γ-Guanidinobutyrate	Glycine amidinotransferase, mitochondrial	GATM	2628	24047826
Guanidinoacetate	Glycine amidinotransferase, mitochondrial	GATM	2628	24047826
Hypoxanthine	Hypoxanthine-guanine phosphoribosyltransferase	HPRT1	3251	26050630
	Purine nucleoside phosphorylase	PNP	4860	24107682
Inosine	5'-nucleotidase	NT5E	4907	25677906
	Cytosolic 5'-nucleotidase 1B	NT5C1B	93034	11690631
	Cytosolic 5'-nucleotidase 1A	NT5C1A	84618	19352542
	5' (3')-deoxyribonucleotidase, cytosolic type	NT5C	30833	15136231
	5' (3')-deoxyribonucleotidase, mitochondrial	NT5M	56953	24506201
	Adenosine deaminase	ADA	56953	24506201
	Cytosolic purine 5'-nucleotidase	NT5C2	22978	25857773
	Adenosine deaminase CECR1	CECR1	51816	25888558
Myoinositol	Alpha-galactosidase A	GLA	2717	25468652
	Inositol monophosphatase 1	IMPA1	3612	11959401
	Inositol monophosphatase 2	IMPA2	3613	21213002
	Glycerophosphodiester phosphodiesterase 1	GDE1	511573	21464471
	Inositol monophosphatase 3	IMPAD1	54928	22887726
N1-Methyl-2-pyridone-5-carboxamide	Aldehyde oxidase	AOX1	316	23857892
Orotic acid	Dihydroorotate dehydrogenase (quinone), mitochondrial	DHODH	1723	23216901
	Uridine 5'-monophosphate synthase	UMPS	7372	22931617
Pseudouridine	Pseudouridine-5'-monophosphatase	HDHD1	617253	19393038
Phenylethylamine	Aromatic-L-amino-acid decarboxylase	DDC	1644	22597765
Sorbitol	Alpha-galactosidase A	GLA	2717	25468652
Thiocyanate	3-mercaptopyruvate sulfurtransferase	MPST	4357	25336638
	Thiosulfate sulfurtransferase	TST	7263	23399736
Thymine	Dihydropyrimidine dehydrogenase (NADP (+))	DPYD	1806	25410891
	Thymidine phosphorylase	TYMP	1890	25304388

Uracil	Dihydropyrimidine dehydrogenase (NADP (+))	DPYD	1806	25410891
	Purine nucleoside phosphorylase	PNP	4860	24107682
	Thymidine phosphorylase	TYMP	1890	25304388
	Uridine phosphorylase 1	UPP1	7378	208568792
	Uridine phosphorylase 2	UPP2	151531	1855639
	Uracil phosphoribosyltransferase homolog	UPRT	139596	17384901
Urea	Arginase-1	ARG1	383	26030248
	Arginase-2, mitochondrial	ARG2	384	26054597
	Agmatinase, mitochondrial	AGMAT	79814	21803059
	Probable allantoicase	ALLC	55821	11054555
Uric acid	Xanthine dehydrogenase/oxidase	XDH	7498	25463089
Uridine	5'-nucleotidase	NT5E	4907	25677906
	Cytosolic 5'-nucleotidase 1B	NT5C1B	93034	11690631
	Cytosolic 5'-nucleotidase 1A	NT5C1A	84618	19352542
Xanthine	Xanthine dehydrogenase/oxidase	XDH	7498	25463089
	Hypoxanthine-guanine phosphoribosyltransferase	HPRT1	3251	26050630
	Guanine deaminase	GDA	9615	16953063
	Purine nucleoside phosphorylase	PNP	4860	24107682
Xanthosine	5'-nucleotidase	NT5E	4907	25677906
	Cytosolic 5'-nucleotidase 1B	NT5C1B	93034	11690631
	Cytosolic 5'-nucleotidase 1A	NT5C1A	84618	19352542
	5' (3')-deoxyribonucleotidase, cytosolic type	NT5C	30833	15136231
	5' (3')-deoxyribonucleotidase, mitochondrial	NT5M	56953	24506201
	Cytosolic purine 5'-nucleotidase	NT5C2	22978	25857773
Hippuric acid	Glycine N-acyltransferase	GLYAT	10249	26149650
Homocysteine	Putative adenosylhomocysteinase 3	AHCYL2	23382	16865262
	Adenosylhomocysteinase	AHCY	191	25248746
	Putative adenosylhomocysteinase 2	AHCYL1	10768	25237103
Hydroquinone	Serum paraoxonase/lactonase 3	PON3	5446	22153698
	Serum paraoxonase/arylesterase 1	PON1	5444	25966589
	Serum paraoxonase/arylesterase 2	PON2	5445	26056385
Indole-3-acetate	4-trimethylaminobutyraldehyde dehydrogenase	ALDH9	223	11790142
	Alpha-aminoadipic semialdehyde dehydrogenase	A1ALDH7	501	26260980
	Aldehyde dehydrogenase, mitochondrial	A1ALDH2	217	26153479
	Fatty aldehyde dehydrogenase	ALDH3A2	224	25784589
	Aldehyde dehydrogenase X, mitochondrial	ALDH1B1	219	21216231
Melatonin	Acetylserotonin O-methyltransferase	ASMT	438	24881886
Methylglyoxal	Aldose reductase	AKR1B1	231	25722213
Putrescine	eroxisomal N (1)-acetyl-spermine/spermidine oxidase	PAOX	196743	20405312
Quinolinic acid	Nicotinate-nucleotide pyrophosphorylase (carboxylating)	QPRT	23475	24038671
Spermidine	Peroxisomal N (1)-acetyl-spermine/spermidine oxidase	PAOX	196743	20405312
	Spermidine synthase	SRM	6723	17585781
	Spermine oxidase	SMOX	54498	25174398

Spermine	Spermine synthase	SMS	6611	23805436
	Spermidine synthase	SRM	6723	17585781
Phenylacetic acid	Aldehyde dehydrogenase, dimeric NADP-preferring	ALDH3A1	218	24762960
	Aldehyde dehydrogenase family 1 member A3	ALDH1A3	220	25684492
	Aldehyde dehydrogenase family 3 member B2	ALDH3B2	222	8890755
	Aldehyde dehydrogenase family 3 member B1	ALDH3B1	221	23721920
Trimethylamine	Flavin Containing Monooxygenase 1	FMO1	2326	25634968
	Flavin Containing Monooxygenase 2	FMO2	2327	25634968
	Flavin Containing Monooxygenase 3	FMO3	2328	25634968
	Flavin Containing Monooxygenase 4	FMO4	2329	25634968
	Flavin Containing Monooxygenase 5	FMO5	2330	25634968

See Figure 2 for the rationale.

Table 7. The receptor complex components for protein-bound uremic toxins and their signal components may also be the key regulators for pathogenic signaling

Protein	Gene	NCBI-Gene ID	PMID
Glycoprotein Gp60	UL1	2657001	26925240
Glycoprotein Gp30	UL44	2952505	26925240
Glycoprotein Gp18	F857_gp18	14182318	26925240
SPARC	P13K	18708	14517321
	Akt	207	
	RhoA*	387	
	SMAD2	4087	
	SMAD3	4088	
	TAK1	7182	
	TAB1	10454	
	MKK4	841591	
FcRn	Numb	8650	25674083
	α -adaptin	101901253	
	CDC42	998	
	RhoA	387	
	PP2A*-	843333	
	Smart2/3	–	
	Ets*	692446	
	c-Jun*	3725	
	Fos*	2353	
	Elk*	131096	
	HIF1*	3091	
	STAT*	6646	
	CREB*	1385	
	Stathmin*	3925	
	PLA2*	5320	

Cubilin-megalin complex	Ets	692446	26055641
	c-Jun	3725	
	Fos	2353	
	Elk	131096	
	HIF1	3091	
	STAT	6646	
	CREB	1385	
	Stathmin	3925	
	PLA2	5320	
RAGE	Bad	572	22934052
	Caspase8	841	
	Gsk3	2932	
	MDM2	4193	
	NF-κB	4790	
	PP2A	843333	
	Ets	692446	
	c-Jun	3725	
	Fos	2353	
	Elk	131096	
	HIF1	3091	
	STAT	6646	
	CREB	1385	
	Stathmin	3925	
	PLA2	5320	
	NFAT	32321	
	Sap1	2539285	
	Max	4149	
	Myc	4609	
	p53	2768677	
	CHOP	1649	
	MEF2	853342	
	ATF-2	1386	

See Figure 2 for the rationale.

diabetes, which were very similar to those observed in metabolic syndrome. Finally, we found that 9 UT genes were upregulated; and another 13 UT genes were slightly downregulated in the pancreas of patients with type 1 diabetes. Taken together, our results suggest that *first*, the upregulation of UT-generating enzymes, protein-bound UT receptors and their signaling components, and convertases for protein/peptide-based UTs in CKD-related diseases at least partially contribute to increased concentrations of UTs; and *second*, some inflammation-modulating genes in UT generation and signaling pathways are upregulated in CKD, CAD and other metabolic diseases, pointing out the potential cross-talking mechanisms underlying the roles of UTs in facilitating CAD in patients with CKD.

4.4. The expressions of uremic toxin genes are modulated by cytokine pathways and regulatory T cells

Our above results indicated that the upregulation of UT-generating enzymes, protein-bound UT receptors and their signaling components, and convertases for protein/peptide-based UTs in CKD-related diseases at least partially contribute to increased concentrations of UTs. The mechanisms underlying this phenomenon are unknown. We hypothesize that elevated UTs in CKD can be sensed

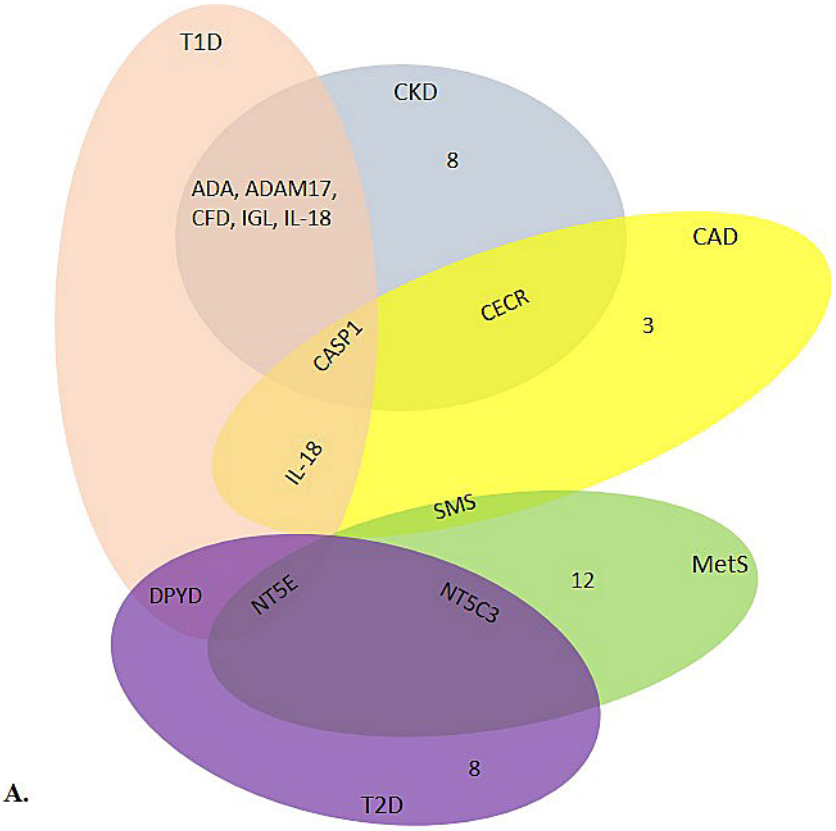
Table 8. 14 out of 34 convertases in the generation of peptide/protein uremic toxins have been identified

Peptide/Protein	Convertase	Convertase gene	Convertase gene ID	PMID
Adiponectin	Furin	FURIN	5045	10433221
	PC7	PCSK7	9159	
Atrial natriuretic peptide	PC1/3	PCSK1	5122	17050541
Calcitonin-gene related peptide (CGRP)	ADAM17	ADAM17	6868	11733179
Clara cell protein	PC1	PCSK1	5122	14608596
	PC2	PCSK2	5125	
	PC5	PCSK5	5126	
Des-acyl ghrelin (DAG)	corin	CORIN	10699	15637153
Endothelin	ECE	ECE1	1899	11067800
Hepcidin	ICE	CASP1	834	8044845
Interleukin-18	caspase-1	CASP1	834	12706898
Interleukin-1β	caspase-1	CASP1	834	12706898
Neuropeptide Y	PC2	PCSK2	5125	7750497
Orexin A	Furin	FURIN	5045	17905609
Parathyroid hormone	ICE	CASP1	834	10449160
Substance P	PC1/3	PCSK1	5122	9405066
	PC2	PCSK2	5125	
Tumor necrosis factor-α	TACE/ADAM17/CD156q	ADAM17	6868	11733179
Adrenomedullin	–	–	–	–
Basic fibroblast growth factor	–	–	–	–
Cholecystokinin	–	–	–	–
Complement factor D	–	–	–	–
Cystatin C	–	–	–	–
Degranulation-inhibiting protein-I, (DIP I)	–	–	–	–
Ghrelin	–	–	–	–
Interleukin-6β	–	–	–	–
Leptin	–	–	–	–
Methionine-enkephalin	–	–	–	–
Motiline	–	–	–	–
Retinol binding protein	–	–	–	–
Uroguanylin	–	–	–	–
Vasoactive intestinal peptide	–	–	–	–
β2-Microglobulin	–	–	–	–
β-Endorphin	–	–	–	–
β-Lipotropin	–	–	–	–
κ-Ig Light chain	–	–	–	–
λ-Ig Light chain	–	–	–	–
δ-Sleep-inducing peptide	–	–	–	–

Table 9. 28 of the 169 genes have been identified in exosomes, which regulate the uremic toxins, the convertase of uremic toxins, the generation enzyme genes and the receptor complex components for protein-bound uremic toxins and their signal components

Chemicals	Gene symbol	Gene ID
Uremic toxins (6)		
β2-Microglobulin	B2M	567
Complement factor D	CFD	1675
Cystatin C	CST3	1471
κ-Ig Light chain	IGK	50820
λ-Ig Light chain	IGL	3535
Retinol binding protein	RBP4	5950
Convertases of uremic toxins (2)		
Furin	FURIN	5045
ECE	ECE1	1899
Enzymes of uremic toxins (18)		
Adenosyl homocysteinase	AHCY	191
Putative adenosylhomocysteinase 2	AHCYL1	10768
Aldose reductase	AKR1B1	231
Aldo-keto reductase family 1 member B10	AKR1B10	57016
Aldehyde dehydrogenase family 3 member B1	ALDH3B1	221
4-trimethylaminobutyraldehyde dehydrogenase	ALDH9A1	223
Aldehyde oxidase	AOX1	316
S-methyl methionine–homocysteine S-methyltransferase	BHMT2	23743
Aromatic-L-amino-acid decarboxylase	DDC	1644
Hypoxanthine-guanine phosphoribosyltransferase	HPRT1	3251
3-mercaptopyruvate sulfurtransferase	MPST	4357
5’ (3’)-deoxyribonucleotidase, cytosolic type	NT5C	30833
5’-nucleotidase	NT5E	4907
Purine nucleoside phosphorylase	PNP	4860
Serum paraoxonase/arylesterase 1	PON1	5444
Serum paraoxonase/lactonase 3	PON3	5446
Nicotinate-nucleotide pyrophosphorylase (carboxylating)	QPRT	23475
Xanthine dehydrogenase/oxidase	XDH	7498
Receptor complex (2)		
FcRn	CASP8	841
RAGE	CDC42	998

by classical DAMP receptor pathways (27, 54). To test this hypothesis, we examined whether the expression of UT genes can be modulated in Toll-like receptor (TLR) pathways. The results showed, in Table 11, that deficiencies of TLR2, TLR3 and TLR4 resulted in decreased expression of a number of genes (3 for TLR2 deficient (TLR2-/-) mice, 4 for TLR3-/- mice and 2 for TLR4-/- mice) as well as increased expression of genes (3 for TLR2-/- mice, 6 for TLR3-/- mice and 4 for TLR4-/- mice). In addition, we also examined a new hypothesis that the expression of UT genes can be modulated via caspase-1-dependent pathways since our reports showed that caspase-1 inflammasome pathways serve as a critical sensor to bridge the risk factors for cardiovascular diseases and initiation of vascular inflammation and atherosclerosis (2, 10, 17, 29, 30). As shown in Table 12, in caspase-1 knockout mice, 12 UT genes were downregulated and 5 UT genes were upregulated, suggesting that caspase-1 pathway plays an important role in promoting UT gene



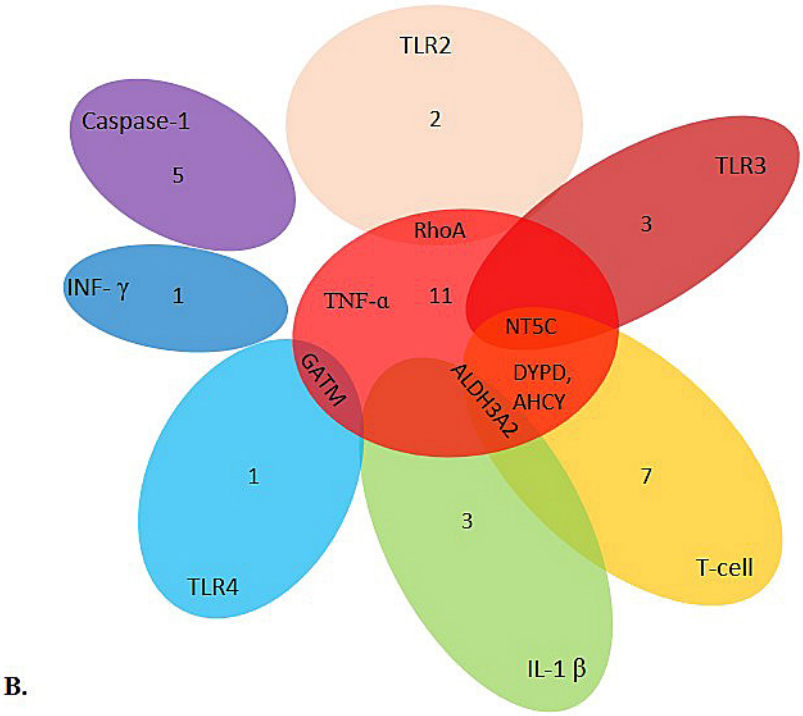


Figure 5. The Venn diagram analyses demonstrate that various diseases may modulate uremic toxin generation in specific or shared manners; and that immune pathways regulate uremic toxin generations in specific or shared manners. A. Not only chronic kidney disease, but also other metabolic diseases upregulate uremic toxins. Chronic kidney disease shares the upregulation of uremic toxins with other metabolic diseases differentially. B. Innate immune sensor cytokines, and adaptive immune cell pathways differentially regulate the generations of uremic toxins.

Table 10. The expressions of the genes encoding uremic toxins, receptor components and toxin-protein conjugation enzymes are more significantly upregulated in metabolic diseases than metabolite-targeted diseases

Disease	Tissue or cell type	Gene	Fold change	Toxin	GEO Dataset ID	PMID
Control VS CKD	Human kidney tubules	**Upregulated gene**			GSE48944	24098934
		ALDH1B	0.72	Indole-3-acetate		
		CALCA	0.82	CGRP		
		CCK	0.86	Cholecystokinin		
		DDC	0.75	Phenylethylamine		
		DHODH	0.72	Orotic acid		
		FMO4	0.57	Trimethylamine		
		GLYAT	0.47	Hippuric acid		
		HAMP	0.80	Hepcidin		
		MDM2	0.86	Signal components		
		NPPA	0.75	Atrial natriuretic peptide		
		NT5M	0.78	Xanthosine/Cytidine		
		PCSK2	0.77	Substance P		
		PON1	0.64	Hydroquinone		
		PON3	0.62	Hydroquinone		
		RBP4	0.36	Retinol binding protein		
		Downregulated gene				
		ADA	1.42	Inosine		
		ADAM17	1.29	CGRP		
		ALDH1A	1.98	Phenylacetic acid		
		B2M	2.31	β2-Microglobulin		
		CASP1	2.31	Interleukin-1β/IL-18		
		CECR1	1.85	Inosine		
		CFD	1.72	Complement factor D		
		FMO3	1.52	Trimethylamine		
		HDHD1	1.53	Pseudouridine		
		IGK	4.56	κ-Ig Light chain		
		IGL	5.38	λ-Ig Light chain		
		IL18	1.65	Interleukin-18		
		PCSK5	1.14	Clara cell protein		
		PON2	1.60	Hydroquinone		
		RHOA	1.84	Signal components		

Control VS Coronary Artery Disease	Human Epicardial Adipose Tissue and Subcutaneous Adipose Tissue)	Upregulated gene			GSE64566	–
		ALDH7A1	0.86	Indole-3-acetate		
		ALDH9A1	0.77	Indole-3-acetate		
		CDC42	0.91	Signal components		
		GAMT	0.92	Creatine		
		HCRT	0.93	Orexin		
		PAOX	0.90	Putrescine/Spermidine		
		PON2	0.88	Hydroquinone		
		PON3	0.89	Hydroquinone		
		TST	0.85	Thiocyanate		
		Downregulated gene				
		ADIPOQ	1.31	Adiponectin		
		AHCYL1	1.10	Homocysteine		
		CASP1	1.15	Interleukin-1β		
		CECR	1.30	Inosine		
		1IL18	1.11	Interleukin-18		
		PCSK7	1.12	Adiponectin		
		SMS	1.10	Spermine		
Control VS Metabolic Syndrome	Human peripheral blood	Upregulated gene			GSE23561	21368773
		HAMP	1.10	Hepcidin		
		NUMB	1.04	Signal components		
		PCSK7	1.14	Adiponectin		
		Downregulated gene				
		AKR1B1	0.97	Arabinitol		
		AKR1B10	0.98	Arabinitol		
		ALDH1B1	0.98	Indole-3-acetate		
		ALDH3B1	0.97	Phenylacetic acid		
		ALDH9A1	0.98	Indole-3-acetate		
		ALLC	0.98	Urea		
		CST3	0.97	Cystatin C		
		GHRL	0.87	Ghrelin		
		HPRT1	0.98	Xanthine/Hypoxanthine		
		NT5C1A	0.98	Uridine/Xanthosine/Cytidine		
		NT5C2	0.98	Cytidine/Inosine/Xanthosine		
		NT5C3	0.98	Cytidine		
		NT5E	0.97	Xanthosine/Cytidine		
		SMOX	0.96	Spermidine		
		SMS	0.97	Spermine		
		UMPS	0.98	Orotic acid		

Uremic toxins are danger patterns or homeostasis patterns.

Control VS Type 2 Diabetes	Human liver	Upregulated gene			GSE23343	21035759
		CALCB	2.14	Calcitonin-gene related peptide (CGRP)		
		HCRT	3.72	Orexin		
		NT5C2	1.42	Cytidine/Inosine/Xanthosine		
		Downregulated gene				
		ADA	0.63	Inosine		
		ADAM17	0.71	CGRP		
		ALDH1A3	0.44	Phenylacetic acid		
		CASP1	0.66	Interleukin-1β/IL-18		
		CDC42	0.68	Signal components		
		CFD	0.60	Complement factor D		
		DPYD	0.68	Thymine/Uracil		
		FMO2	0.50	Trimethylamine		
		IGL	0.47	λ-Ig Light chain		
		IL18	0.33	Interleukin-18		
		MAX	0.45	Signal components		
		MDM2	0.48	Signal components		
		NT5E	0.51	Xanthosine/Cytidine		
Control VS Type 1 Diabetes	Human pancreas	Upregulated gene			GSE72492	–
		ALLC	1.61	Urea		
		ARG1	2.21	Urea		
		GLYAT	1.60	Hippuric acid		
		GUCA2A	1.95	Guanilin		
		NPY	2.53	Neuropeptide Y		
		NT5C1A	1.81	Uridine/Xanthosine/Cytidine		
		PCSK1	4.39	Atrial natriuretic peptide		
		PON1	1.76	Hydroquinone		
		TYMP	1.82	Thymine		
		Downregulated gene				
		AHCY	0.64	Homocysteine		
		ALDH2	0.70	Indole-3-acetate		
		AOX1	0.64	N1-Methyl-2-pyridone-5-carboxamide		
		BHMT2	0.44	Dimethylglycine		
		DPYD	0.67	Thymine/Uracil		
		ECE1	0.73	Endothelin		
		FGF2	0.37	Basic fibroblast growth factor		
		GDE1	0.72	Myoinositol		
		NT5C	0.74	Cytidine/Inosine		
		NT5C3	0.53	Cytidine		
		NT5E	0.52	Uridine/Inosine/Cytidine		

Table 11. DAMPRs/HAMPRs signaling interactions: TLR signaling regulates the expressions of the genes encoding uremic toxins, receptor components and toxin-protein conjugation enzymes

Genotype	Tissue	Gene	Fold Change	Toxin	GEO Database ID	PMID
Control VS TLR2$^{-/-}$	Mus musculus colonic mucosal	Upregulated gene			GSE21845	21228220
		FMO3	1.42	Trimethylamine		
		GAMT	1.16	Creatine		
		PON2	1.14	Hydroquinone		
		GLYAT	1.19	Hippuric acid		
		Downregulated gene				
		PAOX	0.82	Putrescine/Spermidine		
		IMPAD1	0.85	Myoinositol		
		RhoA	0.81	Signal components		
Control VS TLR3$^{-/-}$	Mus musculus liver	Upregulated gene			GSE14719	–
		ALDH9A1	1.14	Indole-3-acetate		
		AGMAT	1.22	Urea		
		ALDH2	1.14	Indole-3-acetate		
		FMO3	1.00	Trimethylamine		
		FMO4	1.54	Trimethylamine		
		PON1	1.15	Hydroquinone		
		DPYD	1.20	Thymine/Uracil		
		AHCY	1.12	Homocysteine		
		Downregulated gene				
		ALDH3B1	0.84	Phenylacetic acid		
		SMOX	0.79	Spermidine		
		NT5C	0.95	Cytidine/Inosine		
		GDA	0.93	Xanthine		
Control VS TLR4$^{-/-}$	Mus musculus kidney	Upregulated gene			GSE34351	22895517
		AGMAT	1.45	Urea		
		ALDH3A2	1.27	Indole-3-acetate		
		DPYD	1.22	Thymine/Uracil		
		ALDH2	1.28	Indole-3-acetate		
		Downregulated gene				
		GATM	0.75	Creatine		
		ALDH1A3	0.57	Phenylacetic acid		

expression, much more than TLR pathways. Moreover, we examined another hypothesis that the expression of UT genes can be modulated by pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), IL-1β, and interferon-γ (IFN-γ) pathways. As shown in Table 13, in TNF-α-treated cells, 13 UT genes were upregulated, and 17 UT genes were downregulated; in IL-1β-treated cells, 2 UT genes were upregulated, and 3 UT genes were downregulated; and in IFN-γ-treated cells, 2 UT genes were upregulated and one UT gene was downregulated. The results suggest that first, caspase-1 pathway plays a more important role in promoting UT gene expression in comparison to other innate immune sensors DAMP receptors; and second, TNF-α pathway plays a more significant role in promoting the expression of UT genes in comparison to other pro-inflammatory cytokine pathways.

Finally, we wanted to examine a new hypothesis that CD4+Foxp3+ regulatory T cells (Tregs), one of the well-characterized immune tolerance cells, since we and others reported that Tregs play a critical role in suppressing vascular inflammation (13–15); and that Tregs are weakened and expanded poorly in CKD patients in hemodialysis (55). As shown in Table 14,

Table 12. The expressions of 17 out of 169 uremic toxin genes are modulated by caspase-1 signal pathways

Gene symbol	Fold change (Caspase-1 KO/WT)	Toxin
Upregulated genes (5)		
XDH	1.22	Uric acid/Xanthine
CST3	1.25	Cystatin C
FMO1	1.32	Trimethylamine
FMO3	1.48	Trimethylamine
LEP	2.9	Leptin
Downregulated genes (12)		
IL1B	0.47	IL-1β
ARG2	0.62	Urea
GDA	0.67	Xanthine
NT5C3	0.68	Cytidine
UPRT	0.69	Uracil
GATM	0.70	γ-Guanidinobutyrate
NT5E	0.73	Cytidine/Inosine/Uridine/Xanthosine
ALDH1A3	0.79	Phenylacetic acid
ALDH3B1	0.79	Phenylacetic acid
UPP1	0.83	Uracil
ALDH9A1	0.85	Indole-3-acetate
RhoA	0.89	Protein binding uremic toxins signal components

in Tregs versus T effector cells, 11 UT genes were upregulated; and 21 UT genes were downregulated. These results suggest that immune suppression mechanism plays an important role in inhibiting the expression of UT genes.

5. DISCUSSION

As technology, including chromatographic methods (ion exchange chromatography, gas chromatography, HPLC), spectrophotometry, fluorometry, chemiluminescence, nephelometry, radioimmunometry, nuclear magnetic resonance and mass spectrometry, has improved, more UTs have been identified (56, 57). This therefore allows for newly identified substances to be added to the list of the European Uremic Toxin (EUTox) Work Group on an ongoing basis, which provides an increasingly complex scenario on their toxicity (56). It has been well documented that some protein/peptide-based UTs, including pro-inflammatory cytokines such as TNF-α, IL-1β, IL-18, and IFN-γ, promote vascular inflammation and other organ inflammation (56). However, the issue of whether host innate immune system uses classical DAMP receptors to sense the elevation of all of other water-soluble and protein-bound UTs remains unknown. It is biochemically difficult for a few classical DAMP receptors, such as TLRs and NLRs, to bind with high affinity to all of those UTs and initiate inflammation efficiently, considering that reduced expression of TLR4 is found in uremic patients (58).

To solve this problem, here, similar to what we reported recently for lysophospholipids, we examined our new hypothesis that UTs can serve as conditional pro-inflammatory DAMPs or anti-inflammatory HAMPs, and that UTs use classical DAMP receptors as well as their intrinsic receptors including RAGE, and serum albumin-toxin receptors to modulate inflammation (54). We have made the following new findings: *1)* Chronic kidney disease selectively accumulates a very small fraction of human serum small-molecule metabolome, roughly 1/80th, as UTs, suggesting that elevation of UTs is highly specific, and may not all result from dysfunctional glomerular filtration; *2)* The serum concentrations of the majority of UTs are increased not only in CKD but also in other diseases, suggesting that some so-called UTs can also be increased when patients have no renal failure; *3)* Protein-bound UTs either induce or suppress the expression of pro-inflammatory molecules rather than only promoting inflammation; *4)* The expression of UT genes is modulated in the proximal tubules of patients with CKD, and adipose tissue of patients with coronary artery disease (CAD), more than in patients with metabolic syndrome and type 2 diabetes, pointing out the potential mechanisms underlying the roles of UTs in accelerating CAD more than other diseases in

Table 13. Pro-inflammation cytokine pathways regulate the expressions of the genes encoding uremic toxins, receptor components and toxin-protein conjugation enzymes

Treatment	Cell type	Gene	Fold Change	Toxin	GEO Dataset ID	PMID
Control VS TNF- α	Annulus disc cells	Upregulated gene			GSE41883	–
		SMOX	6.73	Spermidine		
		AKR1B1	4.66	Arabinitol		
		TYMP	5.70	Thymine		
		PON2	2.06	Hydroquinone		
		NT5E	2.31	Uridine/Xanthosine/Inosine/Cytidine		
		ALDH1B1	2.33	Indole-3-acetate		
		UPP1	4.59	Uracil		
		IMPAD1	1.89	Myoinositol		
		GLA	1.45	Sorbitol/Myoinositol		
		ASMT	1.05	Melatonin		
		GDA	1.04	Xanthine		
		Myc	1.75	Signal components		
		MDM2	1.05	Signal components		
		Downregulated gene				
		ALDH7A1	0.45	Indole-3-acetate		
		DPYD	0.43	Thymine/Uracil		
		NT5C2	0.57	Cytidine/Inosine/Xanthosine		
		ALDH3A2	0.30	Indole-3-acetate		
		ADA	0.43	Inosine		
		GAMT	0.49	Creatine		
		NT5C	0.76	Cytidine/Inosine		
		HPRT1	0.68	Xanthine/Hypoxanthine		
		AHCY	0.73	Homocysteine		
		IMPA2	0.37	Myoinositol		
		GDE1	0.62	Myoinositol		
		SRM	0.75	Spermidine/Spermine		
		CECR1	0.39	Inosine		
		ALDH9A1	0.62	Indole-3-acetate		
		RhoA	0.68	Signal components		
		Max	0.82	Signal components		
		CDC42	0.77	Signal components		
Control VS IL-1β	Human epithelial pancreatic Mia Paca-2 cells	Upregulated gene			GSE26702	22313544
		ALDH2	5.21	Indole-3-acetate		
		AKR1B1	1.44	Arabinitol		
		Downregulated gene				
		ARG2	0.44	Urea		
		FMO5	0.15	Trimethylamine		
		NT5C1B	0.19	Cytidine/Inosine/Uridine		
		ALDH3A2	0.64	Indole-3-acetate		

Control VS IFN-γ	Human hepatocyte	Upregulated gene			GSE38147	22677194
		FMO4	1.63	Trimethylamine		
		GLYAT	5.07	Hippuric acid		
		Max	1.37	Signal components		
		Downregulated gene				
		SMOX	0.52	Spermidine		

Table 14. The expressions of 34 out of 169 uremic toxin genes are modulated in CD4+Foxp3+ regulatory T cells versus in T effector cells

Gene symbol	Fold change	Toxin
Upregulated genes (5)		
Dpyd	1.18	Thymine
Guca2b	1.19	Guanilin
Arg1	1.22	Urea
Npy	1.26	Neuropeptide Y
Casp1	1.30	Interleukin-1β/IL-18
Aldh3a2	1.33	Indole-3-acetate
Adm	1.35	Adrenomedullin
Furin	1.48	Adiponectin/Orexin A
Pon3	1.52	Hydroquinone
Ahcyl2	2.29	Homocysteine
Nt5e	6.35	Xanthosine/Cytidine
Downregulated genes (12)		
Aldh2	0.29	Indole-3-acetate
Fgf2	0.41	Basic fibroblast growth factor
Impa2	0.45	Myoinositol
Umps	0.55	Orotic acid
Ahcy	0.58	Homocysteine
Xdh	0.58	Uric acid
Gla	0.64	Myoinositol/Sorbitol
Aldh7a1	0.64	Indole-3-acetate
Pnp	0.67	Hypoxanthine/Uracil/Xanthine
Paox	0.69	Putrescine
Nt5c2	0.69	Cytidine/Inosine/Xanthosine
Gde1	0.69	Myoinositol
Nt5c3	0.71	Cytidine
Impad1	0.72	Myoinositol
Aldh9a1	0.73	Indole-3-acetate
Mpst	0.77	Thiocyanate
Ece1	0.79	Endothelin
Ada	0.80	Inosine
Impa1	0.81	Myoinositol
Ahcyl1	0.81	Homocysteine
Dhodh	0.84	Orotic acid

patients with CKD; *5)* The expression of UT genes is upregulated by caspase-1-dependent pathway and pro-inflammatory cytokine TNF-α pathways, more than other innate immune sensors, such as TLR pathways, and IL-1β and IFN-γ pathways. This suggests that caspase-1 pathway and TNF-α pathways are potential points of focus for the future development of novel therapy in suppressing UT accelerated pathologies; and *6)* The expression of UT genes is inhibited in Tregs, which emphasizes the importance of Tregs in suppressing the pathogenic effects of UTs (55).

Since 1967, dialysis has been used a standard of care for patients with end-stage renal disease, with numerous new methods being used to complement the dialysis care (59, 60). Dialysis is based on a classical hypothesis that passive accumulation of UTs is due to decreased glomerular filtration in CKD. However, our new findings revealed that UTs represent only 1/80th of human serum small-molecule metabolome, showing that UT accumulation is selective. In addition, considering that roughly 10% of the total metabolites have been identified in human serum metabolome (http://www.serummetabolome.ca), we can further postulate that actually CKD highly selectively accumulates a very tiny fraction of human serum small-molecule metabolome, roughly 1/800th, as UTs (44). Moreover, our results showed that the serum concentrations of the majority of UTs are increased not only in CKD but also in other diseases. Our results suggest that novel anti-caspase-1 and anti-TNF-α therapies and therapeutics in controlling UT-increased diseases together with dialysis could be developed.

Protein-bound UTs are poorly removed by current dialysis techniques because their size is larger than the pore size of dialysis membrane (61). These protein-bound UTs, such as indoxyl sulfate, can induce upregulation of endothelial adhesion molecules, the hallmarks of endothelial cell activation, by binding to human aryl hydrocarbon receptor (AhR) to activate NF-kB and mitogen-activated protein kinases (MAPKs), and NADPH oxidase to increase reactive oxygen species (ROS), both cytosolic ROS and mitochondrial ROS as we recently reported (3, 61, 62). In addition, many UTs bind specifically to the Sudlow's sites I and II of human serum albumin mainly via electrostatic and/or van der Waals forces (63–66). Five types of serum albumin receptors have been identified, including glycoproteins Gp60, Gp30 and Gp18, SPARC, the megalin/cubilin complex, RAGE and the neonatal Fc receptor (FcRn) (51). Moreover, advanced glycation end products (AGE) in UTs can also use RAGE to trigger various intracellular events, such as oxidative stress and inflammation, leading to cardiovascular complications (67, 68). Taken together, our findings suggest that protein-bound UTs may not necessarily use classical DAMP receptors such as TLRs and NLRs to initiate inflammation, may use their intrinsic receptors including AhR, several serum albumin receptors and RAGE to promote inflammation. This conclusion supports our new classification of UTs as conditional danger-associated molecular patterns (DAMPs) or homeostasis-associated molecular patterns (HAMPs). The significance for classifying UTs as conditional DAMPs and HAMPs is that, this model will guide our future work of examining the pathways of new conditional DAMP receptors and HAMP receptors for novel therapeutic purposes.

Recent significant reports and reviews demonstrated a proof of principle that choline, derived by food (dietary) intake from intestine, requires intestinal bacterial enzyme-dependent transformation into trimethylamine (TMA), which is further absorbed into the blood circulation and is transformed into trimethylamine *N*-oxide (TMAO) in host liver by flavin containing monooxygenases (FMOs) (69–71). TMAO is a newly characterized UT that exhibits genetic and dietary regulation, and promotes CKD, cardiovascular disease, impaired glucose intolerance, and atherosclerosis (72–77). We found that the expression of FMO1–5 is modulated by inflammatory pathways, including caspase-1 and TLR pathways. This new finding regarding TMAO generation pathway, together with other results presented in this study as well as other reports, allows us to propose a new working model (Figure 6), which is summarized in the following points of view: First, rather than passive accumulation of endogenous metabolites, a very small fraction of human metabolome, roughly 1/80th of human plasma metabolome, or 1/800th of total human metabolome, eventually becomes selected to be UTs, suggesting that a highly selective mechanism is underlying the generation of UTs; *Second*, the expression of some UT synthases and signaling genes is significantly increased in patients with CKD, CAD and other diseases, suggesting that an increase in UTs; *Third*, the proof of principle demonstrated in TMAO pathway suggests that several factors, including diet, intestinal microbiome, as well as FMOs in host liver all contribute to microbiome-generated UTs; *Fourth*, regulatory T cells and anti-inflammatory cytokines may inhibit the gene expression of UT synthases and signaling pathway components; and *Fifth*, UTs serve as conditional DAMPs and HAMPs, whose intrinsic receptors, in addition to TLRs and NLRs, may initiate UT signaling for regulating vascular inflammation and other inflammatory diseases. These new findings have significantly improved our understanding of molecular mechanisms underlying the roles of UTs in accelerating vascular inflammation, and UT generation, which provide novel insights for the future development of new therapeutics for CKD and CKD-promoted cardiovascular disease and other diseases.

Uremic toxins are danger patterns or homeostasis patterns.

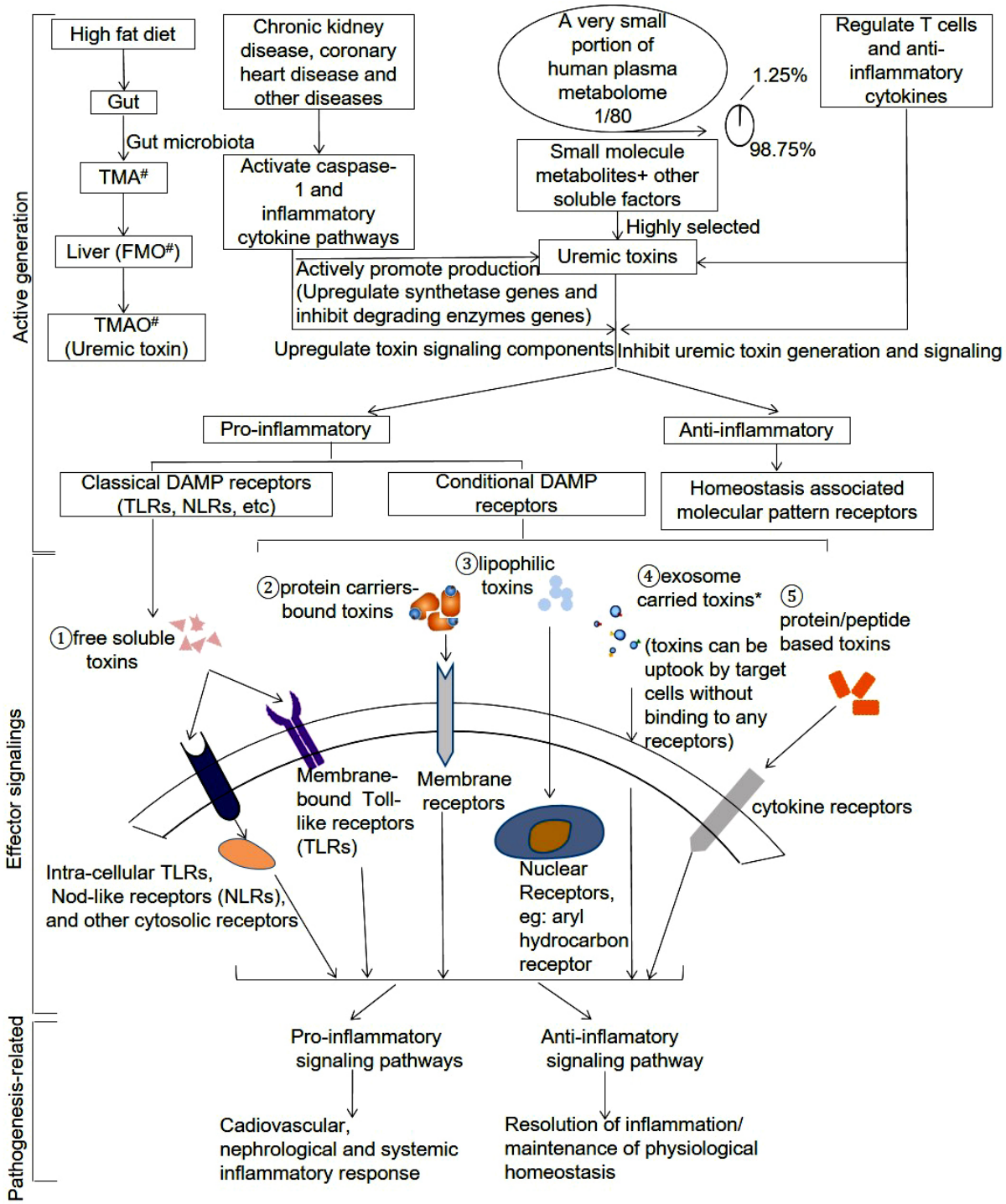


Figure 6. Our new working model: the generations of pathologically uremic toxins can be increased in chromic kidney disease and other inflammatory diseases rather than purely passive accumulation due to failing kidney function. Uremic toxins are conditional danger associated molecular patterns (DAMPs) or homeostasis associated molecular patterns (HAMPs), which are functional in multiple modes in modulating inflammation. The detailed descriptions of this new working model were presented in the Conclusion section of this paper. # TMA: trimethylamine; FMO: Flavin-containing monooxygenase; TMAO: Trimethylamine N-oxide; TLRs: Toll-like receptors; NLRs: Nod-like receptors. Adapted with permission from (Ref number 25143819, 24599232).

6. CONCLUSIONS

Our new findings and others' recent reports allow us to propose a new working model (Figure 6), which is summarized in the following points of view: First, rather than passive accumulation of endogenous metabolites, a very small fraction of human metabolome, roughly 1/80th of human plasma metabolome, or 1/800th of total human metabolome, eventually becomes selected to be UTs, suggesting that a highly selective mechanism is underlying the generation of UTs; Second, the expression of some UT synthases and signaling genes is significantly increased in patients with CKD, CAD and other diseases, suggesting that an increase in UTs; Third, the proof of principle demonstrated in TMAO pathway suggests that several factors, including diet, intestinal microbiome, as well as FMOs in host liver all contribute to microbiome-generated UTs; Fourth, regulatory T cells and anti-inflammatory cytokines may inhibit the gene expression of UT synthases and signaling pathway components; and Fifth, UTs serve as conditional DAMPs and HAMPs, whose intrinsic receptors, in addition to TLRs and NLRs, may initiate UT signaling for regulating vascular inflammation and other inflammatory diseases. These new findings have significantly improved our understanding of molecular mechanisms underlying the roles of UTs in accelerating vascular inflammation, and UT generation, which provide novel insights for the future development of new therapeutics for CKD and CKD-promoted cardiovascular disease and other diseases.

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8. REFERENCES

1. Reikes ST: Trends in end-stage renal disease. Epidemiology, morbidity, and mortality. *Postgrad. Med. J* 108, 124–126, 129–131, 135–126 (2000)

2. Yin Y, Li X, Sha X, Xi H, Li YF, Shao Y, Mai J, Virtue A, Lopez-Pastrana J, Meng S, Tilley DG, Monroy MA, Choi ET, Thomas CJ, Jiang X, Wang H, Yang XF: Early hyperlipidemia promotes endothelial activation via a caspase-1-sirtuin 1 pathway. *Arterioscler Thromb Vasc Biol* 35, 804–816 (2015) DOI: 10.1161/ATVBAHA.115.305282

3. Li X, Fang P, Li Y, Kuo YM, Andrews AJ, Nanayakkara G, Johnson C, Fu H, Shan H, Du F, Hoffman NE, Yu D, Eguchi S, Madesh M, Koch WJ, Sun J, Jiang X, Wang H, Yang X: Mitochondrial reactive oxygen species mediate lysophosphatidylcholine-induced endothelial cell activation. *Arterioscler Thromb Vasc Biol* 36, 1090–1100 (2016) DOI: 10.1161/ATVBAHA.115.306964

4. Fang P, Zhang D, Cheng Z, Yan C, Jiang X, Kruger WD, Meng S, Arning E, Bottiglieri T, Choi ET, Han Y, Yang XF, Wang H: Hyperhomocysteinemia potentiates hyperglycemia-induced inflammatory monocyte differentiation and atherosclerosis. *Diabetes* 63, 4275–4290 (2014) DOI: 10.2337/db14-0809

5. Xi H, Zhang Y, Xu Y, Yang WY, Jiang X, Sha X, Cheng X, Wang J, Qin X, Yu J, Ji Y, Yang X, Wang H: Caspase-1 inflammasome activation mediates homocysteine-induced pyrop-apoptosis in endothelial cells. *Circulation research* 118, 1525–1539 (2016) DOI: 10.1161/CIRCRESAHA.116.308501

6. Ferrer LM, Monroy AM, Lopez-Pastrana J, Nanayakkara G, Cueto R, Li YF, Li X, Wang H, Yang XF, Choi ET: Caspase-1 plays a critical role in accelerating chronic kidney disease-promoted neointimal hyperplasia in the carotid artery. *J Cardiovasc Transl Res* 9, 135–144 (2016) DOI: 10.1007/s12265-016-9683-3

7. Monroy MA, Fang J, Li S, Ferrer L, Birkenbach MP, Lee IJ, Wang H, Yang XF, Choi ET: Chronic kidney disease alters vascular smooth muscle cell phenotype. *Front Biosci (Landmark Ed)* 20, 784–795 (2015) DOI: 10.2741/4337

8. Sha X, Meng S, Li X, Xi H, Maddaloni M, Pascual DW, Shan H, Jiang X, Wang H, Yang XF: Interleukin-35 inhibits endothelial cell activation by suppressing mapk-ap-1 pathway. *J. Biol. Chem* 290, 19307–19318 (2015) DOI: 10.1074/jbc.M115.663286

9. Shao Y, Cheng Z, Li X, Chernaya V, Wang H, Yang XF: Immunosuppressive/ anti-inflammatory cytokines directly and indirectly inhibit endothelial dysfunction–a

novel mechanism for maintaining vascular function. *J Hematol Oncol* 7, 80 (2014)
DOI: 10.1186/s13045-014-0080-6

10. Lopez-Pastrana J, Ferrer LM, Li YF, Xiong X, Xi H, Cueto R, Nelson J, Sha X, Li X, Cannella AL, Imoukhuede PI, Qin X, Choi ET, Wang H, Yang XF: Inhibition of caspase-1 activation in endothelial cells improves angiogenesis: A novel therapeutic potential for ischemia. *J. Biol. Chem* 290, 17485–17494 (2015)
DOI: 10.1074/jbc.M115.641191

11. Zhang CE, Wei W, Liu YH, Peng JH, Tian Q, Liu GP, Zhang Y, Wang JZ: Hyperhomocysteinemia increases beta-amyloid by enhancing expression of gamma-secretase and phosphorylation of amyloid precursor protein in rat brain. *J. Pathol* 174, 1481–1491 (2009)

12. Zhang D, Fang P, Jiang X, Nelson J, Moore JK, Kruger WD, Berretta RM, Houser SR, Yang X, Wang H: Severe hyperhomocysteinemia promotes bone marrow-derived and resident inflammatory monocyte differentiation and atherosclerosis in ldlr/cbs-deficient mice. *Circulation research* 111, 37–49 (2012)
DOI: 10.1161/CIRCRESAHA.112.269472

13. Xiong Z, Song J, Yan Y, Huang Y, Cowan A, Wang H, Yang XF: Higher expression of bax in regulatory t cells increases vascular inflammation. *Front Biosci* 13, 7143–7155 (2008)
DOI: 10.2741/3217

14. Xiong Z, Yan Y, Song J, Fang P, Yin Y, Yang Y, Cowan A, Wang H, Yang XF: Expression of tctp antisense in cd25 (high) regulatory t cells aggravates cuff-injured vascular inflammation. *Atherosclerosis* 203, 401–408 (2009)
DOI: 10.1016/j.atherosclerosis.2008.07.041

15. Yang WY, Shao Y, Lopez-Pastrana J, Mai J, Wang H, Yang XF: Pathological conditions re-shape physiological tregs into pathological tregs. *Burns & trauma* 3, 1 (2015)

16. Nelson J, Wu Y, Jiang X, Berretta R, Houser S, Choi E, Wang J, Huang J, Yang X, Wang H: Hyperhomocysteinemia suppresses bone marrow cd34+/vegf receptor 2+ cells and inhibits progenitor cell mobilization and homing to injured vasculature-a role of beta1-integrin in progenitor cell migration and adhesion. *FASEB J* 29, 3085–3099 (2015)
DOI: 10.1096/fj.14-267989

17. Li YF, Huang X, Li X, Gong R, Yin Y, Nelson J, Gao E, Zhang H, Hoffman NE, Houser SR, Madesh M, Tilley DG, Choi ET, Jiang X, Huang CX, Wang H, Yang XF: Caspase-1 mediates hyperlipidemia-weakened progenitor cell vessel repair. *Front Biosci (Landmark Ed)* 21, 178–191 (2016)
DOI: 10.2741/4383

18. Shao Y, Chernaya V, Johnson C, Yang WY, Cueto R, Sha X, Zhang Y, Qin X, Sun J, Choi ET, Wang H, Yang XF: Metabolic diseases downregulate the majority of histone modification enzymes, making a few upregulated enzymes novel therapeutic targets-"sand out and gold stays". *J Cardiovasc Transl Res* 9, 49–66 (2016)
DOI: 10.1007/s12265-015-9664-y

19. Kahn MR, Robbins MJ, Kim MC, Fuster V: Management of cardiovascular disease in patients with kidney disease. Nature reviews. *Cardiology* 10, 261–273 (2013)
DOI: 10.1038/nrcardio.2013.15

20. Schiffrin EL, Lipman ML, Mann JF: Chronic kidney disease: Effects on the cardiovascular system. *Circulation* 116, 85–97 (2007)
DOI: 10.1161/
CIRCULATIONAHA.106.678342

21. Moe SM, Chen NX: Pathophysiology of vascular calcification in chronic kidney disease. *Circ Res* 95, 560–567 (2004)
DOI: 10.1161/01.RES.0000141775.67189.98

22. Mestas J, Ley K: Monocyte-endothelial cell interactions in the development of atherosclerosis. *Trends Cardiovasc Med* 18, 228–232 (2008)
DOI: 10.1016/j.tcm.2008.11.004

23. Moradi H, Sica DA, Kalantar-Zadeh K: Cardiovascular burden associated with uremic toxins in patients with chronic kidney disease. *Am. J. Nephrol* 38, 136–148 (2013)
DOI: 10.1159/000351758

24. Apostolov EO, Basnakian AG, Ok E, Shah SV: Carbamylated low-density lipoprotein: Nontraditional risk factor for cardiovascular events in patients with chronic kidney disease. *J Ren Nutr* 22, 134–138 (2012)
DOI: 10.1053/j.jrn.2011.10.023

25. Speer T, Owala FO, Holy EW, Zewinger S, Frenzel FL, Stahli BE, Razavi M, Triem S, Cvija H, Rohrer L, Seiler S, Heine GH, Jankowski V, Jankowski J, Camici GG, Akhmedov A, Fliser D, Luscher TF, Tanner FC: Carbamylated low-density lipoprotein induces endothelial dysfunction. *Eur Heart J* 35, 3021–3 (2014)
DOI: 10.1093/eurheartj/ehu111

26. Mehta JL, Basnakian AG: Interaction of carbamylated ldl with lox-1 in the induction of endothelial dysfunction and atherosclerosis. *Eur Heart J* 35, 2996–7 (2014)
DOI: 10.1093/eurheartj/ehu122

27. Yang XF, Yin Y, Wang H: Vascular inflammation and atherogenesis are activated via receptors for pamps and suppressed by regulatory t cells. *Drug Discov Today Ther Strateg* 5, 125–142 (2008)
DOI: 10.1016/j.ddstr.2008.11.003

28. Venereau E, Ceriotti C, Bianchi ME: Damps from cell death to new life. *Front Immunol* 6, 422 (2015)
DOI: 10.3389/fimmu.2015.00422

29. Yin Y, Pastrana JL, Li X, Huang X, Mallilankaraman K, Choi ET, Madesh M, Wang H, Yang XF: Inflammasomes: Sensors of metabolic stresses for vascular inflammation. *Front Biosci (Landmark Ed)* 18, 638–649 (2013)
DOI: 10.2741/4127

30. Yin Y, Yan Y, Jiang X, Mai J, Chen NC, Wang H, Yang XF: Inflammasomes are differentially expressed in cardiovascular and other tissues. *Int J Immunopathol Pharmacol* 22, 311–322 (2009)
DOI: 10.1177/039463200902200208

31. Wang L, Fu H, Nanayakkara G, Li Y, Shao Y, Johnson C, Cheng J, Yang WY, Yang F, Lavallee M, Xu Y, Cheng X, Xi H, Yi J, Yu J, Choi ET, Wang H, Yang X: Novel extracellular and nuclear caspase-1 and inflammasomes propagate inflammation and regulate gene expression: A comprehensive database mining study. *J Hematol Oncol* 9, 122 (2016)
DOI: 10.1186/s13045-016-0351-5

32. Yin Y, Pastrana JL, Li X, Huang X, Mallilankaraman K, Choi ET, Madesh M, Wang H, Yang XF: Inflammasomes: Sensors of metabolic stresses for vascular inflammation. *Front Biosci* 18, 638–649 (2013)
DOI: 10.2741/4127

33. Wang X, Li YF, Nanayakkara G, Shao Y, Liang B, Cole L, Yang WY, Li X, Cueto R, Yu J, Wang H, Yang XF: Lysophospholipid receptors, as novel conditional danger receptors and homeostatic receptors modulate inflammation-novel paradigm and therapeutic potential. *J Cardiovasc Transl Res* 9, 343–359 (2016)
DOI: 10.1007/s12265-016-9700-6

34. Huang X, Gong R, Li X, Virtue A, Yang F, Yang IH, Tran AH, Yang XF, Wang H: Identification of novel pretranslational regulatory mechanisms for nf-kappab activation. *J. Biol. Chem* 288, 15628–15640 (2013)
DOI: 10.1074/jbc.M113.460626

35. Neirynck N, Vanholder R, Schepers E, Eloot S, Pletinck A, Glorieux G: An update on uremic toxins. *Int Urol Nephrol* 45, 139–150 (2013)
DOI: 10.1007/s11255-012-0258-1

36. Niwa T: Update of uremic toxin research by mass spectrometry. *Mass Spectrom. Rev* 30, 510–521 (2011)
DOI: 10.1002/mas.20323

37. Vanholder R, Glorieux G, De Smet R, Lameire N: New insights in uremic toxins. *Kidney Int Suppl* S6–10 (2013)
DOI: 10.1046/j.1523-1755.63.s84.43.x

38. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G: Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A* 106, 3698–3703 (2009)
DOI: 10.1073/pnas.0812874106

39. Koyama Y, Takeishi Y, Arimoto T, Niizeki T, Shishido T, Takahashi H, Nozaki N, Hirono O, Tsunoda Y, Nitobe J, Watanabe T, Kubota I: High serum level of pentosidine, an advanced glycation end product (age), is a risk factor of patients with heart failure. *J Card Fail* 13, 199–206 (2017)
DOI: 10.1016/j.cardfail.2006.11.009

40. Virtue A, Wang H, Yang XF: Micrornas and toll-like receptor/interleukin-1 receptor signaling. *J Hematol Oncol* 5, 66 (2012)
DOI: 10.1186/1756-8722-5-66

41. Sladek FM: What are nuclear receptor ligands? *Mol. Cell. Endocrinol* 334, 3–13 (2011)
DOI: 10.1016/j.mce.2010.06.018

42. Velenosi TJ, Feere DA, Sohi G, Hardy DB, Urquhart BL: Decreased nuclear receptor activity and epigenetic modulation associates with down-regulation of hepatic drug-metabolizing enzymes in chronic kidney disease. *FASEB J* 28, 5388–5397 (2014)
DOI: 10.1096/fj.14-258780

43. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L: Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int* 78, 838–848 (2010)
DOI: 10.1038/ki.2010.278

44. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, Young N, Xia J, Knox C, Dong E, Huang P, Hollander Z, Pedersen TL, Smith SR, Bamforth F, Greiner R, McManus B, Newman JW, Goodfriend T, Wishart DS: The human serum metabolome. *PloS one* 6, e16957 (2011)
DOI: 10.1371/journal.pone.0016957

45. Miyamoto Y, Iwao Y, Mera K, Watanabe H, Kadowaki D, Ishima Y, Chuang VT, Sato K, Otagiri M, Maruyama T: A uremic toxin, 3-carboxy-4-methyl-5-propyl-2-furanpropionate induces cell damage to proximal tubular cells via the generation of a radical intermediate. Biochem. *Pharmacol* 84, 1207–1214 (2012)
DOI: 10.1016/j.bcp.2012.07.033

46. Niwa T, Takeda N, Yoshizumi H: Rna metabolism in uremic patients: Accumulation of modified ribonucleosides in uremic serum. Technical note. *Kidney Int* 53, 1801–1806 (1998)
DOI: 10.1046/j.1523-1755.1998.00944.x

47. Vanholder R, De Smet R, Lesaffer G: P-cresol: A toxin revealing many neglected but relevant aspects of uraemic toxicity. *Nephrol Dial Transplant* 14, 2813–2815 (1999)
DOI: 10.1093/ndt/14.12.2813

48. Mandal S, Mandal A, Johansson HE, Orjalo AV, Park MH: Depletion of cellular polyamines, spermidine and spermine, causes a total arrest in translation and growth in mammalian cells. *Proc Natl Acad Sci U S A* 110, 2169–2174 (2013)
DOI: 10.1073/pnas.1219002110

49. Viaene L, Annaert P, de Loor H, Poesen R, Evenepoel P, Meijers B: Albumin is the main plasma binding protein for indoxyl sulfate and p-cresyl sulfate. *Biopharm Drug Dispos* 34, 165–175 (2013)
DOI: 10.1002/bdd.1834

50. Merlot AM, Kalinowski DS, Richardson DR: Unraveling the mysteries of serum albumin-more than just a serum protein. *Front Physiol* 5, 299 (2014)
DOI: 10.3389/fphys.2014.00299

51. Larsen MT, Kuhlmann M, Hvam ML, Howard KA: Albumin-based drug delivery: Harnessing nature to cure disease. *Mol Cell Ther* 4, 3 (2016)
DOI: 10.1186/s40591-016-0048-8

52. Sparvero LJ, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, Rutledge R, Lin B, Amoscato AA, Zeh HJ, Lotze MT: Rage (receptor for advanced glycation endproducts), rage ligands, and their role in cancer and inflammation. *J. Transl. Med* 7, 17 (2009)
DOI: 10.1186/1479-5876-7-17

53. Gonzalez-Calero L, Martin-Lorenzo M, Alvarez-Llamas G: Exosomes: A potential key target in cardio-renal syndrome. *Front Immunol* 5, 465 (2014)
DOI: 10.3389/fimmu.2014.00465

54. Wang X, Li YF, Nanayakkara G, Shao Y, Liang B, Cole L, Yang WY, Li X, Cueto R, Yu J, Wang H, Yang XF: Lysophospholipid receptors, as novel conditional danger receptors and homeostatic receptors modulate inflammation-novel paradigm and therapeutic potential. *J Cardiovasc Transl Res* 9, 343–59 (2016)
DOI: 10.1007/s12265-016-9700-6

55. Afzali B, Edozie FC, Fazekasova H, Scotta C, Mitchell PJ, Canavan JB, Kordasti SY, Chana PS, Ellis R, Lord GM, John S, Hilton R, Lechler RI, Lombardi G: Comparison of regulatory t cells in hemodialysis patients and healthy controls: Implications for cell therapy in transplantation. *Clin J Am Soc Nephro: CJASN* 8, 1396–1405 (2013)
DOI: 10.2215/CJN.12931212

56. Lisowska-Myjak B: Uremic toxins and their effects on multiple organ systems. *Nephron. Clinical practice* 128, 303–311 (2014)
DOI: 10.1159/000369817

57. Tanaka H, Sirich TL, Plummer NS, Weaver DS, Meyer TW: An enlarged profile of uremic solutes. *PloS one* 10, e0135657 (2015)
DOI: 10.1371/journal.pone.0135657

58. Ando M, Shibuya A, Tsuchiya K, Akiba T, Nitta K: Reduced capacity of mononuclear cells to synthesize cytokines against an inflammatory stimulus in uremic patients. *Nephron. Clinical practice* 104, c113–119 (2006)
DOI: 10.1159/000094446

59. Klarenbach SW, Tonelli M, Chui B, Manns BJ: Economic evaluation of dialysis therapies. Nature reviews. *Nephrology* 10, 644–652 (2014)
DOI: 10.1038/nrneph.2014.145

60. Glorieux G, Tattersall J: Uraemic toxins and new methods to control their accumulation: Game changers for the concept of dialysis adequacy. *Clin Kidney J* 8, 353–362 (2015)
DOI: 10.1093/ckj/sfv034

61. Ito S, Yoshida M: Protein-bound uremic toxins: New culprits of cardiovascular events in chronic kidney disease patients. *Toxins* 6, 665–678 (2014)
DOI: 10.3390/toxins6020665

62. Sallee M, Dou L, Cerini C, Poitevin S, Brunet P, Burtey S: The aryl hydrocarbon receptor-activating effect of uremic toxins from tryptophan metabolism: A new concept to understand cardiovascular complications of chronic kidney disease. *Toxins* 6, 934–949 (2014)
DOI: 10.3390/toxins6030934

63. Sudlow G, Birkett DJ, Wade DN: The characterization of two specific drug binding sites on human serum albumin. *Mol. Pharmacol* 11, 824–832 (1975)

64. Takamura N, Maruyama T, Otagiri M: Effects of uremic toxins and fatty acids on serum protein binding of furosemide: Possible mechanism of the binding defect in uremia. *Clin. Chem* 43, 2274–2280 (1997)

65. Jourde-Chiche N, Dou L, Cerini C, Dignat-George F, Vanholder R, Brunet P: Protein-bound toxins–update 2009. *Semin Dial* 22, 334–339 (2009)
DOI: 10.1111/j.1525-139X.2009.00576.x

66. Vanholder R, Hoefliger N, De Smet R, Ringoir S: Extraction of protein bound ligands from azotemic sera: Comparison of 12 deproteinization methods. *Kidney Int* 41, 1707–1712 (1992)
DOI: 10.1038/ki.1992.244

67. Jourde-Chiche N, Dou L, Cerini C, Dignat-George F, Brunet P: Vascular incompetence in dialysis patients–protein-bound uremic toxins and endothelial dysfunction. *Semin Dial* 24, 327–337 (2011)
DOI: 10.1111/j.1525-139X.2011.00925.x

68. Stinghen AE, Massy ZA, Vlassara H, Striker GE, Boullier A: Uremic toxicity of advanced glycation end products in ckd. *JASN* 27, 354–370 (2016)
DOI: 10.1681/ASN.2014101047

69. Fennema D, Philips IR, Shephard EA: Trimethylamine and trimethylamine n-oxide, a flavin-containing monooxygenase 3 (fmo3)-mediated host-microbiome metabolic axis implicated in health and disease. *Drug Metab Dispos* 44, 1839–1850 (2016)
DOI: 10.1124/dmd.116.070615

70. Aron-Wisnewsky J, Clement K: The gut microbiome, diet, and links to cardiometabolic and chronic disorders. Nature reviews. *Nephrology* 12, 169–181 (2016)
DOI: 10.1038/nrneph.2015.191

71. Obeid R, Awwad HM, Rabagny Y, Graeber S, Herrmann W, Geisel J: Plasma trimethylamine n-oxide concentration is associated with choline, phospholipids, and methyl metabolism. *Am J Clin Nutr* 103, 703–711 (2016)
DOI: 10.3945/ajcn.115.121269

72. Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, Allayee H, Lee R, Graham M, Crooke R, Edwards PA, Hazen SL, Lusis AJ: Trimethylamine-n-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab* 17, 49–60 (2013)
DOI: 10.1016/j.cmet.2012.12.011

73. Tang WH, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatisa-Boyle B, Li XS, Levison BS, Hazen SL: Gut microbiota-dependent trimethylamine n-oxide (tmao) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circulation research* 116, 448–455 (2015)
DOI: 10.1161/CIRCRESAHA.116.305360

74. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA,

Lusis AJ, Hazen SL: Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472, 57–63 (2011)
DOI: 10.1038/nature09922

75. Gao X, Liu X, Xu J, Xue C, Xue Y, Wang Y: Dietary trimethylamine n-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. *J Biosci Bioeng* 118, 476–481 (2014)
DOI: 10.1016/j.jbiosc.2014.03.001

76. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, DiDonato AJ, Fu X, Hazen JE, Krajcik D, DiDonato JA, Lusis AJ, Hazen SL: Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* 163, 1585–1595 (2015)
DOI: 10.1016/j.cell.2015.11.055

77. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL: Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med* 19, 576–585 (2013)
DOI: 10.1038/nm.3145

Abbreviations: DAMP: danger signal-associated molecular patterns; HAMP: homeostasis-associated molecular patterns; CKD: chronic kidney disease; UT: uremic toxins; CAD: coronary artery disease; TNF-α: Tumor necrosis factor α; TLR: toll-like receptors; IL-1β: Interleukin-1 beta; IFN-γ: Interferon-gamma; EC: endothelial cell; ESRD: end-stage renal disease; GFR: glomerular filtration rate; BUN: blood urea nitrogen; cLDL: carbamylated LDL; VSMC: vascular smooth muscle cell; PAMP: pathogen-associated molecular patterns; NLR: NOD (nucleotide binding and oligomerization domain)-like receptors; RAGE: advanced glycation end products; MCP-1: monocyte chemoattractant protein-1; A2AR: adenosine A2A receptor; A2R: adenosine A2 receptor; IL-18R: interleukin-18 receptor; IL6R: interleukin 6 receptor; IL-1R: Interleukin-1 receptor; LEPR/OBR: Leptin receptor; MT1/MT2: Melatonin receptor; RZR/ROR: orphan receptor; RAGE: receptor for advanced glycation endproduct; TNFR: tumor necrosis factor receptor; AHR: Aryl hydrocarbon receptor; GPR35: G protein-coupled receptor 35; GP85/CD44: CD44 antigen; Octβ2R: the beta adrenergic-like octopamine receptor; GPCR: G-protein-coupled receptors; NK-1R, NK-2R, NK-3R: Neurokinin-1 receptor, Neurokinin-2 receptor, Neurokinin-3 receptor; CRLR: calcitonin receptor-like receptor; CALCRL: Calcitonin receptor-like; RAMP1: Receptor activity modifying protein 1; GHSR1a: Growth hormone secretagogue receptor; OX1R/OX2R: Orexin receptor type 1,Orexin receptor type 2; PTH1R: parathyroid hormone 1 receptor; GC-C, GC-D: receptor-guanylate cyclase; VPAC1, VPAC2: vasoactive intestinal peptide (VIP) receptor; AdipoR1, AdipoR2: Adiponectin receptor; NPR1, NPR2, NPR3: Atrial natriuretic peptide receptor; bFGF-R1, bFGF-R2: basic fibroblast growth factor receptors; CCK1R, CCK2R: cholecystokinin receptor; ETA, ETB1, ETB2, ETC: endothelin receptors; NPY1R, NPY2R, NPY3R, NPY4R, NPY5R, NPY6R: Neuropeptide Y receptors

Key Words: Uremia, Uremic Toxins, Danger Signal-Associated Molecular Patterns, Homeostasis-Associated Molecular Patterns, DAMPs, HAMPs, DAMP and HAMP receptors, Inflammation

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