

# FEATURED NEW INVESTIGATOR

## REVIEW ARTICLE

### Mesencephalic astrocyte-derived neurotrophic factor (MANF), a new player in endoplasmic reticulum diseases: structure, biology, and therapeutic roles



YEAWON KIM, SUN-JI PARK, and YING MAGGIE CHEN

ST. LOUIS, MO

**Mesencephalic astrocyte-derived neurotrophic factor (MANF), a newly identified 18-kDa soluble protein, localizes to the luminal endoplasmic reticulum (ER), whose stress can stimulate MANF expression and secretion. In *Drosophila* and zebrafish, MANF regulates dopaminergic neuron development. In contrast, in mice, MANF deficiency leads to diabetes and activation of the unfolded protein response. Recent studies in rodent models have demonstrated that MANF mitigates diabetes, exerts neurotrophic function in neurodegenerative disease, protects cardiomyocytes and neurons in myocardial infarction and cerebral ischemia, respectively, and promotes immune cell phenotype switch from proinflammatory macrophages to prorepair anti-inflammatory macrophages. The cytoprotective mechanisms of MANF on ER stress are currently under active investigation. In addition, for the first time, we have discovered that MANF can potentially serve as a urinary ER stress biomarker in ER stress-mediated kidney disease. These studies have underscored the diagnostic and therapeutic importance of MANF in ER diseases. (Translational Research 2017;188:1–9)**

**Abbreviations:** AAV = adeno-associated virus; AKI = acute kidney injury; ARMET = arginine-rich mutated in early tumors; ATF6 = activating transcription factor 6; BiP = Ig-binding protein; CDNF = conserved dopamine neurotrophic factor; CHOP = C/EBP homologous protein; DM = diabetes mellitus; ER = endoplasmic reticulum; ERSE = ER stress response element; ERSR = ER stress response; GDNF = glial cell line-derived neurotrophic factor; I/R = ischemia/reperfusion; IRE1 = inositol-requiring enzyme-1; KDELR = KDEL receptor; LAMB2 = laminin  $\beta$ 2; MANF = mesencephalic astrocyte-derived neurotrophic factor; MAPK = mitogen-activated protein kinase;

**Ying Maggie Chen, MD, PhD,** is an Assistant professor of Medicine in the Division of Nephrology at the Washington University School of Medicine. Her article is based on a presentation given at the annual meeting of the Central Society for Clinical and Translational Research (CSCTR) in 2015. She also received an Early Career Development Award from CSCTR in 2015. Dr. Chen's research focuses on the molecular pathogenesis of endoplasmic reticulum (ER) stress-mediated kidney diseases with the goal of discovering ER stress-associated biomarkers and developing targeted clinical therapies to treat ER stress-associated kidney diseases. From the Division of Nephrology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Mo.

Submitted for publication March 4, 2017; revision submitted June 20, 2017; accepted for publication June 23, 2017.

Reprint requests: Ying Maggie Chen, Washington University School of Medicine, Division of Nephrology, Campus Box 8126 660 S. Euclid Ave., St. Louis, MO 63110; e-mail: [ychen@dom.wustl.edu](mailto:ychen@dom.wustl.edu).

1931-5244/\$ - see front matter

© 2017 Elsevier Inc. All rights reserved.

<http://dx.doi.org/10.1016/j.trsl.2017.06.010>

MCAO = middle cerebral artery occlusion; MI = myocardial infarction; NF = nuclear transcriptional factor; NMR = Nuclear magnetic resonance; NOD = nonobese diabetic; NS = nephrotic syndrome; 6-OHDA = 6-hydroxydopamine; PD = Parkinson's disease; PDGF = platelet-derived growth factor; PERK = PKR-like ER kinase; Pvf-1 = PDGF- and vascular endothelial growth factor-related factor-1; SAP domain = SAF-A/B, Acinus and PIAS domain; SCA = spinocerebellar ataxia; SCG = superior cervical ganglion; sl = simulated ischemia; sl/R = simulated ischemia/reperfusion; SN = substantia nigra; T1D = type 1 diabetes; TBP = TATA box-binding protein; TG = thapsigargin; TM = tunicamycin; UPR = unfolded protein response; XBP1s = splicing XBP1

## INTRODUCTION

The endoplasmic reticulum (ER) is the central site for folding, post-translational modifications, and transport of secretory and membrane proteins. Environmental, aging, and genetic factors disrupting ER function lead to accumulation of misfolded and unfolded proteins in the ER lumen, termed ER stress.<sup>1</sup> The adaptive response to ER stress is the unfolded protein response (UPR), which is initiated by 3 ER transmembrane proteins, inositol-requiring enzyme-1 (IRE1), PKR-like ER kinase, and activating transcription factor 6 (ATF6).<sup>2</sup> IRE1 is a dual activity enzyme with an endoribonuclease and a kinase domain. IRE1-mediated cleavage of XBP1 leads to a spliced XBP1 mRNA (XBP1s), encoding a potent transcriptional activator.<sup>3</sup> IRE1 also recruits tumor necrosis factor receptor-associated factor 2 and activates Jun N-terminal kinase, thus transmitting apoptotic signals.<sup>4</sup> The PKR-like ER kinase phosphorylates the eukaryotic initiation factor-2 $\alpha$ , leading to attenuation of protein translation and induction of activating transcription factor 4.<sup>5</sup> ATF6 activates transcription of chaperones after being cleaved by S1P/S2P proteases in Golgi from 90 kDa to the active 50-kDa ATF6 (p50ATF6).<sup>6</sup> Cells rendered dysfunctional due to severe or prolonged ER stress are eliminated from the organism by the proapoptotic UPR, mediated by C/EBP homologous protein, Jun N-terminal kinase, or caspase 12 (Fig 1). Dysregulation of the UPR pathway can result in various diseases such as diabetes, inflammation, and neurodegenerative disorders including Alzheimer's disease and Parkinson's disease (PD), which are collectively known as "ER diseases."<sup>8</sup> Mounting evidence has also demonstrated that ER stress contributes to the development and progression of renal glomerular and tubular diseases.<sup>9</sup>

Podocyte ER stress and dysfunction induced by podocyte gene mutations have been shown to play an important role in the pathogenesis of proteinuria. In cell culture studies, certain nephrin or podocin missense mutants are trapped inside the ER and activate ER stress.<sup>10-12</sup> Moreover, in mouse models, podocyte ER stress induced by *LAMB2* C321R,<sup>13</sup> *ACTN4* K256E,<sup>14</sup> or *COL4A3* G1334E<sup>15</sup> leads to podocytopathy. Meanwhile, in diverse sporadic nephropathies including focal segmental glomerulosclerosis, membranous nephropathy, minimal change disease, and diabetic nephropathy,

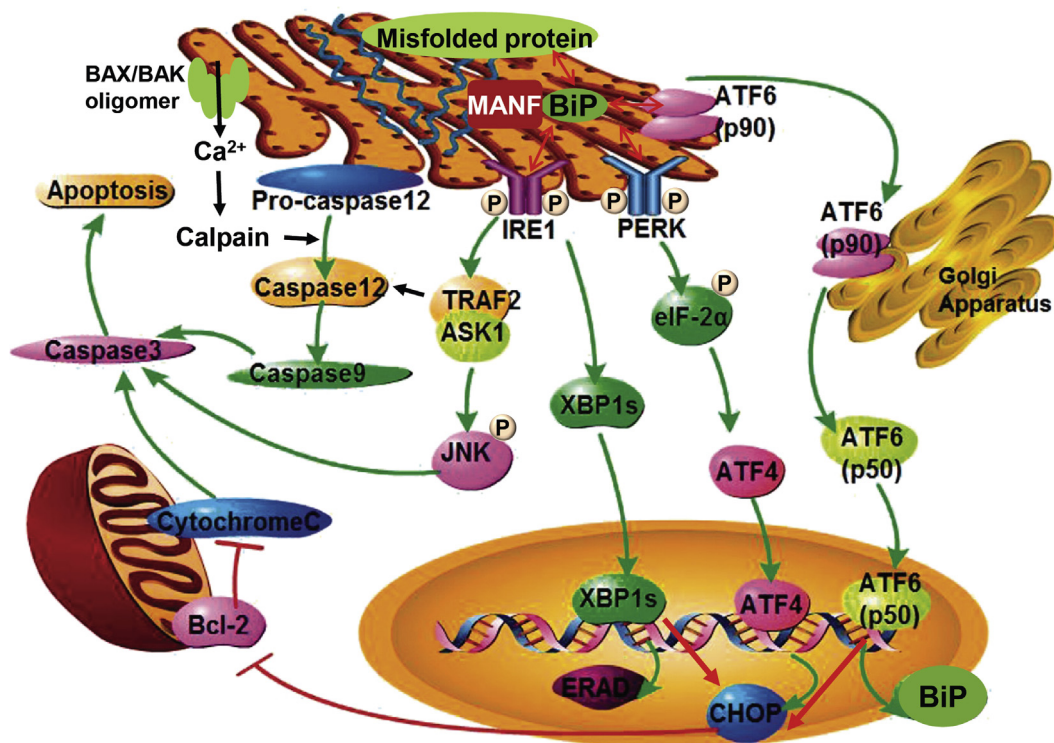
multiple studies have linked podocyte ER stress to the pathogenesis of these diseases in both experimental models<sup>16-18</sup> and human kidney biopsies.<sup>19,20</sup> Similarly, in renal tubular cells, ER dysfunction induces tubular injury and apoptosis and plays a pathogenic role in the onset and progression of renal tubulointerstitial disease, which ultimately advances to end-stage renal disease.<sup>21-25</sup>

Mesencephalic astrocyte-derived neurotrophic factor (MANF), also known as arginine-rich mutated in early tumors, or ARMET, was first discovered by Dr Commissiong's group in 2003 as a new dopaminergic neurotrophic factor in astrocyte-conditioned medium.<sup>26</sup> Emerging studies have demonstrated that MANF is a novel ER-soluble protein and that its cytoprotective effects are not limited to neurons. In the current review, we will provide an up-to-date overview about the molecular structure of MANF, molecular mechanisms regulating MANF upregulation and secretion, important functions of MANF in different disease models, utility of MANF as an ER stress biomarker, and its translational potential.

## MANF STRUCTURE

Cloning and sequencing of the MANF cDNA verified that MANF encoded by a 4.3 kb gene with 4 exons is located on human chromosome 3.<sup>26</sup> The MANF primary transcript encompasses 1109 bp which codes for a predicted 179-amino acid protein. The N-terminal 21 amino acids serve as the signal peptide, targeting nascent MANF to the ER. The secreted form of MANF is the full-length protein without the signal sequence, that is, 158 amino acids with a molecular weight of 18 kDa.<sup>27</sup> Nuclear magnetic resonance spectroscopy of the 3-dimensional solution structure of human MANF reveals that MANF is composed of an N-terminal saposin-like domain (residues 1–95) and a C-terminal SAP (SAF-A/B, Acinus and PIAS) domain (residues 104–158), which are connected with a short linker (residues 96–103)<sup>28</sup> (Fig 2).

The N-terminus of MANF can bind membrane and free lipids.<sup>29</sup> Importantly, the C-terminus is homologous to the SAP domain of Ku70, a well-known inhibitor of proapoptotic Bax protein.<sup>28</sup> It has been shown that the small C-terminal domain is responsible for the intracellular



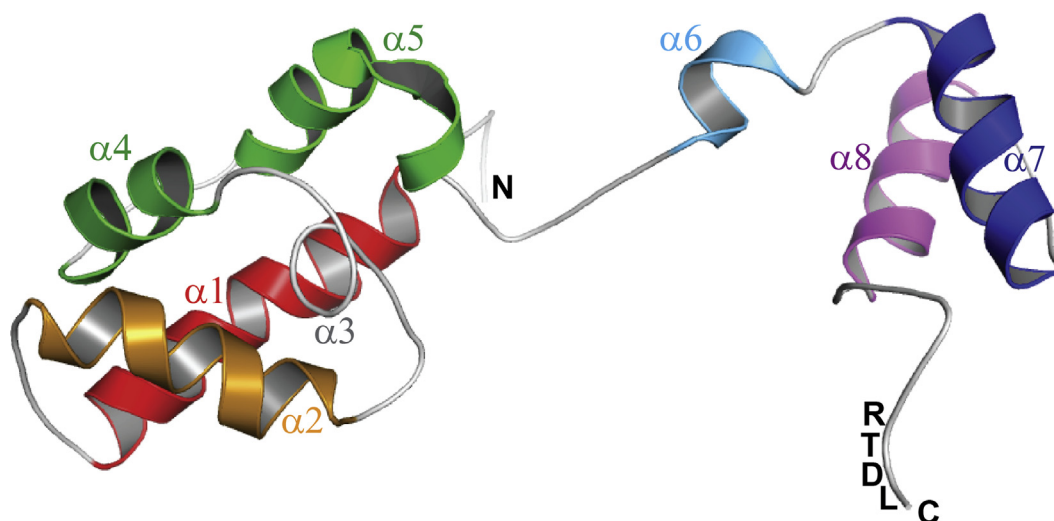
**Fig 1.** The UPR pathway and the possible interaction between MANF and BiP. UPR, unfolded protein response; MANF, mesencephalic astrocyte-derived neurotrophic factor; BiP, Ig-binding protein; IRE1, inositol-requiring enzyme-1; TRAF-2, tumor necrosis factor receptor-associated factor 2; JNK, Jun N-terminal kinase; PERK, PKR-like ER kinase; eIF-2 $\alpha$ , eukaryotic initiation factor-2 $\alpha$ ; XBP1s, splicing XBP1; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; CHOP, C/EBP homologous protein. The image is modified from Xu et al.<sup>7</sup> with permission.

protection against Bax-dependent apoptosis in the sympathetic superior cervical ganglion neurons.<sup>28</sup> It has also been shown that the C-terminal SAP-like domain of MANF interacts with the DNA binding domain of NF- $\kappa$ B p65 subunit after translocation of MANF into nucleus under inflammation and ER stress, which negatively regulates NF- $\kappa$ B signaling.<sup>30</sup> MANF contains 8 conserved cysteines that form 4 intramolecular disulfide bonds including 2 in CXXC sequences.<sup>29</sup> Although the CXXC motif can be found in thiol-disulfide oxidoreductase,<sup>27,31</sup> MANF is not involved in redox reactions. The CKGC disulfide bridge in the MANF C-terminus is crucial for MANF's neuroprotective activity.<sup>31</sup> In addition, the 4 amino acids, RTDL at the extreme C-terminus of MANF (Fig 2) can bind to the KDEL receptors (KDELRs), which are localized primarily in the *cis*-Golgi facilitate the cycling of MANF from the Golgi to the ER.<sup>31,32</sup> In vitro deletion of RTDL in superior cervical ganglion neurons almost completely abrogates the intracellular prosurvival effect of MANF by causing it to localize to the Golgi complex.<sup>31</sup> Interestingly, the interaction between RTDL and KDELRs can

also be found at the plasma membrane of SH-SY5Y cells, which is required for MANF binding at cell surface.<sup>32</sup>

### MANF EXPRESSION AND UPREGULATION UPON ER STRESS

MANF mRNA and protein are widely expressed in both neurons and non-neuronal tissues. In the brain, relatively high MANF levels are detected in the cerebral cortex, hippocampus, and cerebellar Purkinje cells. In non-neuronal tissues, high MANF expression is detected in the adult liver, testis, and salivary gland.<sup>33</sup> In vitro MANF expression is upregulated in cell lines derived from bone tissue (U2OS), kidneys (HEK293), neuroblastoma (SH-SY5Y), and embryo fibroblasts (NIH 3T3) in response to ER stress triggered by tunicamycin (TM), thapsigargin (TG), and lactatystin.<sup>27,34</sup> In vivo MANF expression is induced in chondrocytes following ER retention of the mutant cartilage extracellular matrix protein in mouse knock-in models of chondrodysplasia caused by matrilin-3 or type X collagen mutations,<sup>35</sup> in pancreatic  $\beta$  cells following misfolding of mutant proinsulin in Akita mouse model



**Fig 2.** NMR solution structure of human MANF. NMR, nuclear magnetic resonance; MANF, mesencephalic astrocyte-derived neurotrophic factor.

carrying an insulin C96Y mutation,<sup>27</sup> and in synoviocytes following inflammation-mediated ER stress in a rabbit antigen-induced arthritis model.<sup>30</sup> MANF expression is also increased in response to ischemia-induced ER stress both in vitro and in vivo.<sup>33,34,36-38</sup>

Related to ER stress-mediated kidney diseases, we have shown that in our podocyte ER stress-induced hereditary nephrotic syndrome (NS) mouse model in which podocyte ER stress is activated by the C321R mutation of the glomerular basement membrane constituent laminin  $\beta$ 2 (LAMB2), MANF is induced and secreted by ER-stressed podocytes.<sup>13,39</sup> We have also shown that in acute kidney injury triggered by ER stressor TM or ischemia/reperfusion (I/R), MANF is upregulated and secreted by ER-stressed tubules.<sup>39</sup>

MANF expression has also recently been studied in human diseases involving ER stress. Chen LJ. et al. reports that compared with healthy controls, MANF mRNA is dramatically upregulated in peripheral white blood cells from patients with autoimmune inflammatory disease, including rheumatoid arthritis and systemic lupus erythematosus.<sup>30</sup> Galli E. et al. shows that high serum MANF concentrations are found in children 1–9 years of age close to the diagnosis of type 1 diabetes (T1D), but not in older children and adolescents 10–17 years of age with recent-onset T1D or adults with long-term T1D.<sup>40</sup> The increased MANF concentrations in 1- to 9-year-old patients with T1D are inversely correlated with C-peptide levels that are an indirect measure of functional  $\beta$  cell mass, but not associated with diabetes-predictive autoantibodies.<sup>40</sup> These results suggest that elevated serum MANF level at the onset of T1D may reflect ER stress in the remaining  $\beta$  cells strained with increased demand for insulin production due to reduced  $\beta$  cell mass.

ER stress-induced transcriptional upregulation of MANF is driven by an ER stress response element (ERSE)-II in the MANF promoter, which is immediately downstream of  $-160$  bp in the promoter.<sup>27,37</sup> ERSE-II (ACGTGGNCCAAT) contains 2 transcriptional factor recognition sequences: ACGTGG is recognized by ATF6 or XBP1, whereas CCAAT is recognized by nuclear transcriptional factor (NF)-Y. It has been shown that both binding sites are required for MANF induction by ER stress.<sup>27</sup> Moreover, ATF6 $\alpha$  most strongly increases the MANF promoter activity via ERSE-II, while the effects of ATF6 $\beta$  and XBP1s are moderate in Neuro2a cells.<sup>41</sup> In agreement with these reports, MANF expression is upregulated in the hearts of ATF6 transgenic mice that constitutively express a tamoxifen-activated form of ATF6 in cardiomyocytes.<sup>42</sup>

#### ER STRESS-ENHANCED MANF SECRETION

Trafficking and secretion of MANF is regulated by ER stress. Enhanced protein secretion is not a general cellular response to ER stress. However, a robust secretory response of MANF to TG-induced ER stress, but not to TM or DTT, was observed in SH-SY5Y, cardiomyocytes, and HeLa cells.<sup>32,43</sup> MANF secretion is influenced by its N- and C-terminal sequences. The signal peptide directs MANF to the ER during protein synthesis and allows access to the secretory pathway.<sup>33</sup> The C-terminal sequence RTDL of MANF (Fig 2), unlike most ER stress response (ERSR) proteins that contain the canonical ER retention signal KDEL, functions as an ER retention signal by binding to the KDEL receptor in the Golgi. Removal of the RTDL sequence promotes MANF secretion,<sup>32,43,44</sup> whereas KDEL receptor overexpression reduces MANF secretion.<sup>32</sup> However, RTDL has a lower affinity



**Table I.** Protective functions of MANF in various rodent models of ER diseases

---

1. Diabetes	<ul style="list-style-type: none"> <li>• AAV-mediated MANF overexpression in pancreas attenuates diabetes in a diabetic mouse model<sup>46</sup></li> </ul>
2. Neurodegenerative disease	<ul style="list-style-type: none"> <li>• Intrastrially injected MANF restores the function of the nigrostriatal dopaminergic system in a rat model of Parkinson's disease caused by 6-OHDA<sup>47</sup></li> <li>• MANF overexpression in cerebellum ameliorates spinocerebellar ataxia in a genetic mouse model arising from mutant TATA box-binding protein-mediated Purkinje cell degeneration<sup>48</sup></li> </ul>
3. Ischemia	<ul style="list-style-type: none"> <li>• Intracerebral administration of MANF before or after ischemic insult reduces infarct volume and promotes behavioral recovery in a rat model of cerebral ischemia induced by ipsilateral middle cerebral artery occlusion<sup>49-51</sup></li> <li>• Infusion of recombinant MANF before cardiac ischemia protects the heart from ischemic damage<sup>43</sup></li> </ul>
4. Retina degeneration	<ul style="list-style-type: none"> <li>• Subretinal injection of recombinant MANF at the onset of retinal degeneration enhances the efficiency of retinal regenerative therapies in the mouse model<sup>52</sup></li> </ul>

---

*Abbreviations:* AAV, adeno-associated virus; MANF, mesencephalic astrocyte-derived neurotrophic factor; 6-OHDA, 6-hydroxydopamine.

for the receptor than KDEL in averting secretion. Thus, under nonstressed conditions, MANF is efficiently retained in the ER and not secreted. In contrast, on ER stress, ERSR proteins with both KDEL and RTDL are induced while KDEL levels do not change. It has been proposed that due to the differential affinities of canonical and noncanonical ER retention sequences for the KDEL, proteins with KDEL motifs compete better for binding to the KDEL than MANF with RTDL motif, thus fostering MANF secretion.<sup>45</sup> There are opposite findings regarding whether the RTDL motif regulates TG-induced MANF secretion. Henderson et al. showed an RTDL-dependent induction of MANF secretion in response to TG in SH-SY5Y cells.<sup>32</sup> On the contrary, Glembotski et al. found an RTDL-independent secretory response of MANF to Tg in cardiac myocytes and HeLa cells.<sup>43</sup> The composition and levels of KDEL in different cell types may account for the observed differences, which remains to be resolved. Meanwhile, MANF secretion is also regulated by Ig-binding protein (BiP) via calcium-dependent interaction of MANF and BiP in the ER (Fig 1).<sup>43</sup> BiP overexpression inhibits secretion of MANF, independently of the C-terminal RTDL.<sup>43,44</sup> In addition, the BiP-MANF complex is TG-sensitive and the complex levels decrease in coordination with ER calcium depletion.<sup>43</sup> Finally, Oh-hashi et al. reported that MANF secretion is almost completely abolished by the overexpression of a dominant-negative Sar1 by impairing COPII-mediated transport from the ER to Golgi, indicating that the secretion of MANF is COPII pathway dependent.<sup>44</sup>

#### MANF FUNCTIONS (TABLE I) AND UNDERLYING MECHANISMS

**MANF deficiency and diabetes mellitus (DM).** To investigate the physiological role of MANF in vivo, Lindahl et al. generated MANF global knockout mice (*Manf*<sup>-/-</sup>).

In contrast to neuronal phenotypes observed in the knockout of *Manf* in *Drosophila*<sup>53</sup> and knockdown in zebrafish,<sup>54</sup> *Manf*<sup>-/-</sup> mice developed DM postnatally.<sup>46</sup> The diabetic phenotype is due to the progressive reduction of  $\beta$  cell mass resulting from decreased proliferation and increased apoptosis.<sup>46</sup> Moreover, MANF ablation in vivo leads to generalized activation of the UPR in  $\beta$  cells.<sup>46</sup> Differently from the insulin-deficient phenotype of *Manf*<sup>-/-</sup> mice, a MANF gene homozygous mutation (*IVS1 + 1G > T*) in a human patient presents with type 2 diabetes and obesity.<sup>55</sup> In line with these studies, using a transgenic T1D nonobese diabetic mouse model that expresses hen egg lysozyme in  $\beta$  cells and thus increases in the basal load of unfolded protein stress, a recent study has shown that reduced islet expression of transcription factor *Glis3* caused by genetic variation or a high-fat diet and the resultant defect in *Manf* upregulation led to enhanced susceptibility to apoptosis and  $\beta$ -cell failure.<sup>56</sup> The same transcriptional relationships are also observed in human islets.<sup>56</sup> Remarkably, recombinant human MANF induces  $\beta$ -cell proliferation in vitro and overexpression of MANF by adeno-associated virus serotype 6-mediated transduction in the pancreas of diabetic mice promotes  $\beta$ -cell regeneration.<sup>46</sup> Thus, MANF could be a therapeutic candidate to treat DM.

**Neurotrophic function in neurodegenerative diseases.** Parkinson's disease (PD) is a progressive degenerative brain disease characterized by bradykinesia, resting tremor, muscle rigidity, and postural abnormalities. The movement disorder is mainly a consequence of degeneration of dopamine neurons projecting from the substantia nigra to the caudate-putamen (dorsal striatum). MANF and conserved dopamine neurotrophic factor form a novel, evolutionarily conserved neurotrophic factor family<sup>57</sup> and are structurally distinct from other neurotrophic factors including glial cell line-derived neurotrophic factor and neurturin. Low to

intermediate levels of MANF are expressed in the striatum and substantia nigra.<sup>33</sup> In vitro, MANF was shown to selectively protect nigral dopaminergic neurons, but not GABAergic or serotonergic neurons.<sup>26</sup> In *Drosophila*, loss of the MANF ortholog leads to a deficiency in dopaminergic neuron development, which can be rescued by fly and human MANF.<sup>53</sup> In a rat model of PD induced by 6-hydroxydopamine (6-OHDA) that caused degeneration of dopamine neurons, intrastrially injected MANF restores the function of the nigrostriatal dopaminergic system when administered either 6 hours before or 4 weeks after 6-OHDA administration in the striatum. It was hypothesized that the neurotrophic effect of MANF against 6-OHDA-induced neurotoxicity may be attributable to inhibition of ER stress.<sup>47</sup>

MANF is also enriched in cerebellar Purkinje cells.<sup>33</sup> In a genetic mouse model of spinocerebellar ataxia (SCA) arising from mutant TATA box-binding protein-mediated Purkinje cell degeneration (SCA17 knock-in mice), MANF expression is reduced in the mutant mouse cerebellum.<sup>48</sup> More importantly, overexpression of MANF either by cerebellum injection of lentivirus-expressing MANF or by transgenic MANF expression specifically in the central nervous system ameliorates severe loss of Purkinje neurons and ataxic gait in SCA17 mice, which is dependent on protein kinase C signaling.<sup>48</sup>

**Protection against cerebral and cardiac ischemia.** Brain ischemia induces neuron death through ER stress.<sup>58,59</sup> It has been shown that in a rat model of focal cerebral ischemia induced by ipsilateral middle cerebral artery occlusion (MCAO), MANF protein is significantly induced in ischemic cortical neurons at the early stage after ischemia, which occurs before C/EBP homologous protein activation and histologic evidence of infarction.<sup>34,38</sup> Under focal cerebral ischemia, MANF expression is also upregulated in rat glial cells, including astrocytes.<sup>36</sup> Similarly, following global forebrain ischemia caused by bilateral occlusion of the common carotid arteries in combination with hypotension, MANF mRNA is increased in the hippocampus and cerebral cortex.<sup>33</sup> In vitro study shows that human recombinant MANF inhibits ER stress-induced apoptosis in cultured primary neurons.<sup>38</sup> More importantly, in vivo studies demonstrate that intracerebral injection of human recombinant MANF 20 minutes before MCAO or intracortical delivery of adeno-associated virus serotype 7 encoding human MANF 1 week before MCAO in rats reduces infarct volume and facilitates motor recovery.<sup>49,50</sup> The neuroprotective effect of MANF is mediated, at least in part, through attenuation of ischemia-mediated cell apoptosis.<sup>50</sup> In addition, ipsilateral ventricle injection of human recombinant MANF 2 hours after MCAO

rescues the cerebral ischemia-induced neuron loss and promotes behavioral recovery in rats, which is associated with the inhibition of ischemia-induced upregulation of ERSR proteins including BiP, phosphorylated IRE1, and XBP1s, and suppression of caspase 3 cleavage.<sup>51</sup>

Myocardial infarction also activates ER stress in the surviving myocytes adjacent to the damaged region in vivo and simulated ischemia (sI) or simulated ischemia/reperfusion (sI/R) induces ERSR in cultured rat and mouse ventricular myocytes in vitro.<sup>42,60,61</sup> Correlating with ER stress induction, MANF expression is shown to be upregulated in cardiac myocytes of mice subjected to myocardial infarction in vivo and in cultured neonatal rat ventricular myocytes subjected to sI in an ATF6 and XBP1-dependent manner in vitro.<sup>37</sup> miRNA-mediated knocking down of endogenous MANF in cultured cardiac myocytes increases cell death on sI/R, whereas overexpression of MANF by adenovirus or addition of recombinant MANF to medium protects cardiac myocytes from serum starvation or sI- or sI/R-mediated cell death.<sup>37</sup> Furthermore, infusion of recombinant MANF into mice 24 hours before subjecting them to 30 minutes of myocardial ischemia has cardioprotective effect.<sup>43</sup>

**Immune modulation and inhibition of inflammation.** Immune modulation by MANF plays an important role in the repair and regeneration of retina. Regenerative therapies based on cell replacement hold promise for the treatment of a range of degenerative retina diseases, such as macular degeneration or retinitis pigmentosa. However, the proinflammatory microenvironments negatively affect integration and repair. A recent study has shown that in *Drosophila* or mouse, the damaged retina secretes Pvf-1 (platelet-derived growth factor (PDGF)- and vascular endothelial growth factor-related factor-1) and PDGF-A, respectively. These damage signals induce MANF expression in innate immune cells and MANF activation promotes a phenotype switch of macrophages from proinflammatory to anti-inflammatory, thereby improving tissue repair in both invertebrates and vertebrates, and thus enhancing retinal regenerative therapy.<sup>52</sup>

MANF can also repress the proliferation of inflammatory fibroblast-like synoviocytes in a rodent methylated bovine serum albumin-induced arthritis model.<sup>30</sup> Furthermore, MANF can alleviate oxygen-glucose deprivation-induced cell damage and inflammation in rat primary astrocytes,<sup>62</sup> as well as suppress lipopolysaccharide-induced inflammatory response in neural stem cells.<sup>63</sup>

The molecular mechanisms by which MANF attenuates inflammation remain to be fully understood. Recent data demonstrate that MANF in the nucleus interferes

with the binding of NF- $\kappa$ B p65 subunit to its target gene promoters and subsequently suppresses the expression of NF- $\kappa$ B-dependent inflammatory genes.<sup>30</sup> MANF also inhibits p38 mitogen-activated protein kinase pathway.<sup>63</sup>

#### UTILITY OF MANF AS AN ER STRESS BIOMARKER

Although ER stress underpins the initiation and/or development of a variety of ER diseases, ER stress biomarkers that can be applied in human patients are still lacking to date. For the first time, we demonstrate that MANF can potentially serve as a urinary biomarker for detecting ER stress in podocytes or renal tubular cells, respectively.<sup>39</sup>

In the podocyte ER stress-induced NS mouse model that we have developed, transgenic expression of C321R-LAMB2 in podocytes via the podocyte-specific mouse nephrin promoter on the *Lamb2*<sup>-/-</sup> background (Tg-C321R mice) recapitulates the features of human NS patients carrying the C321R mutation. As a control, transgenic expression of wild-type LAMB2 in podocytes is sufficient to rescue the NS phenotype in *Lamb2*<sup>-/-</sup> mice.<sup>13,64</sup> In the first postnatal month, when Tg-C321R mutants exhibit trace proteinuria without notable renal histologic alternations, podocyte ER stress as manifested by the upregulation of BiP and MANF that is induced by the C321R misfolded protein is evident. Most importantly, MANF is easily detected in urine specimens from C321R mutants at the incipient stage of NS, but not in the controls. In addition, urinary MANF excretion increases during disease progression in the C321R mutants.<sup>39</sup>

Similarly, in the acute kidney injury mouse model triggered by TM or I/R, significant upregulation of MANF at both transcriptional and translational levels is observed in the ER-stressed renal tubules before obvious renal histopathologic changes or elevation of serum creatinine occur. Moreover, urinary MANF excretion concurrent with tubular cell ER stress precedes histologic or clinical manifestations of the corresponding disease, respectively.<sup>39</sup>

Our study will help identify subgroups of patients associated with ER dysfunction who can be treated with ER stress modulators in a highly-targeted manner. We will continue to validate MANF as a urinary ER stress biomarker in human patients with ER stress-mediated kidney diseases.

#### CONCLUSION

In summary, MANF, a novel ER-soluble protein, is upregulated and secreted in response to experimental ER stress in various cell and rodent models. Accumulating evidence has demonstrated important protective and immunomodulatory effects of MANF in different

disease models. Thus, MANF may have significant therapeutic potential to treat ER stress-mediated diseases including kidney disease. To date, knowledge about the intracellular and extracellular actions of MANF is still limited and the receptor for MANF remains to be identified. Further studies are required to dissect the MANF downstream signaling pathways, investigate how MANF regulates the UPR, and validate whether MANF can be utilized as a noninvasive ER stress biomarker in human ER diseases.

#### ACKNOWLEDGMENTS

Conflicts of Interest: A patent application entitled “Mesencephalic astrocyte-derived neurotrophic factor (MANF) as a urine biomarker for endoplasmic reticulum (ER) stress-related kidney disease, methods and uses therefore” has been filed by Y.M. Chen and Washington University Office of Technology Management (application number 14730465, filed on June 4, 2015). All authors have read the journal’s policy on disclosure of potential conflicts of interest.

Y.M. Chen is supported by the National Institutes of Health K08DK089015, R03DK106451, and R01DK105056A1, Halpin Foundation-American Society of Nephrology Research Grant, Faculty Scholar Award (MD-FR-2013-336) from the Children’s Discovery Institute of Washington University and St. Louis Children’s Hospital, Clinical Scientist Development Award (Grant 2015100) from the Doris Duke Charitable Foundation, Career Development Award from Nephrotic Syndrome Study Network (NEPTUNE), Early Career Development Award from Central Society for Clinical and Translational Research (CSCTR), and Renal Translational Innovation Grant from Washington University Division of Nephrology. Y.M. Chen is a member of Washington University Diabetes Research Center (supported by NIH P60 DK020579), Washington University Musculoskeletal Research Center (supported by NIH P30AR057235), and Washington University Institute of Clinical and Translational Sciences (UL1 TR000448).

The authors would like to acknowledge Drs. Adam Scott and Li Ding for preparation of Fig 2.

No editorial support was received in the preparation of the manuscript. All authors have read the journal’s authorship agreement, and the manuscript has been reviewed and approved by all named authors.

#### REFERENCES

1. Osowski CM, Urano F. Measuring ER stress and the unfolded protein response using mammalian tissue culture system. *Methods Enzymol* 2011;490:71–92.
2. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; 8:519–29.

3. Yoshida H, Oku M, Suzuki M, Mori K. pXBP1(U) encoded in XBP1 pre-mRNA negatively regulates unfolded protein response activator pXBP1(S) in mammalian ER stress response. *J Cell Biol* 2006;172:565–75.
4. Urano F, Wang X, Bertolotti A, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 2000;287:664–6.
5. Harding HP, Novoa I, Zhang Y, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* 2000;6:1099–108.
6. Ye J, Rawson RB, Komuro R, et al. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 2000;6:1355–64.
7. Xu Y, Guo M, Jiang W, et al. Endoplasmic reticulum stress and its effects on renal tubular cells apoptosis in ischemic acute kidney injury. *Ren Fail* 2016;38:831–7.
8. Yoshida H. ER stress and diseases. *Febs J* 2007;274:630–58.
9. Inagi R, Ishimoto Y, Nangaku M. Proteostasis in endoplasmic reticulum—new mechanisms in kidney disease. *Nat Rev Nephrol* 2014;10:369–78.
10. Drozdova T, Papillon J, Cybulsky AV. Nephropathy missense mutations: induction of endoplasmic reticulum stress and cell surface rescue by reduction in chaperone interactions. *Physiol Rep* 2013;1:e00086.
11. Fan Q, Zhang H, Ding J, et al. R168H and V165X mutant podocin might induce different degrees of podocyte injury via different molecular mechanisms. *Genes Cells* 2009;14:1079–90.
12. Liu L, Done SC, Khoshnoodi J, et al. Defective nephropathy trafficking caused by missense mutations in the NPHS1 gene: insight into the mechanisms of congenital nephrotic syndrome. *Hum Mol Genet* 2001;10:2637–44.
13. Chen YM, Zhou Y, Go G, et al. Laminin beta2 gene missense mutation produces endoplasmic reticulum stress in podocytes. *J Am Soc Nephrol* 2013;24:1223–33.
14. Cybulsky AV, Takano T, Papillon J, et al. Glomerular epithelial cell injury associated with mutant alpha-actinin-4. *Am J Physiol Ren Physiol* 2009;297:F987–95.
15. Pieri M, Stefanou C, Zaravinos A, et al. Evidence for activation of the unfolded protein response in collagen IV nephropathies. *J Am Soc Nephrol* 2014;25:260–75.
16. Cybulsky AV, Takano T, Papillon J, et al. Complement C5b-9 membrane attack complex increases expression of endoplasmic reticulum stress proteins in glomerular epithelial cells. *J Biol Chem* 2002;277:41342–51.
17. Cybulsky AV, Takano T, Papillon J, Bijjan K. Role of the endoplasmic reticulum unfolded protein response in glomerular epithelial cell injury. *J Biol Chem* 2005;280:24396–403.
18. Inoki K, Mori H, Wang J, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *J Clin Invest* 2011;121:2181–96.
19. Bek MF, Bayer M, Muller B, et al. Expression and function of C/EBP homology protein (GADD153) in podocytes. *Am J Pathol* 2006;168:20–32.
20. Markan S, Kohli HS, Joshi K, et al. Up regulation of the GRP-78 and GADD-153 and down regulation of Bcl-2 proteins in primary glomerular diseases: a possible involvement of the ER stress pathway in glomerulonephritis. *Mol Cell Biochem* 2009;324:131–8.
21. Bando Y, Tsukamoto Y, Katayama T, et al. ORP150/HSP12A protects renal tubular epithelium from ischemia-induced cell death. *FASEB J* 2004;18:1401–3.
22. Kawakami T, Inagi R, Wada T, et al. Indoxy sulfate inhibits proliferation of human proximal tubular cells via endoplasmic reticulum stress. *Am J Physiol Ren Physiol* 2010;299:F568–76.
23. Khan MA, Liu J, Kumar G, et al. Novel orally active epoxyeicosatrienoic acid (EET) analogs attenuate cisplatin nephrotoxicity. *FASEB J* 2013;27:2946–56.
24. Lindenmeyer MT, Rastaldi MP, Ikehata M, et al. Proteinuria and hyperglycemia induce endoplasmic reticulum stress. *J Am Soc Nephrol* 2008;19:2225–36.
25. Ohse T, Inagi R, Tanaka T, et al. Albumin induces endoplasmic reticulum stress and apoptosis in renal proximal tubular cells. *Kidney Int* 2006;70:1447–55.
26. Petrova P, Raibekas A, Pevsner J, et al. MANF: a new mesencephalic, astrocyte-derived neurotrophic factor with selectivity for dopaminergic neurons. *J Mol Neurosci* 2003;20:173–88.
27. Mizobuchi N, Hoseki J, Kubota H, et al. ARMET is a soluble ER protein induced by the unfolded protein response via ERSE-II element. *Cell Struct Funct* 2007;32:41–50.
28. Hellman M, Arumae U, Yu LY, et al. Mesencephalic astrocyte-derived neurotrophic factor (MANF) has a unique mechanism to rescue apoptotic neurons. *J Biol Chem* 2011;286:2675–80.
29. Parkash V, Lindholm P, Peranen J, et al. The structure of the conserved neurotrophic factors MANF and CDFN explains why they are bifunctional. *Protein Eng Des Sel* 2009;22:233–41.
30. Chen LJ, Feng LJ, Wang X, et al. Mesencephalic astrocyte-derived neurotrophic factor is involved in inflammation by negatively regulating the NF-kappa B pathway. *Sci Rep-uk* 2015;5:8133.
31. Matlik K, Yu LY, Eesmaa A, et al. Role of two sequence motifs of mesencephalic astrocyte-derived neurotrophic factor in its survival-promoting activity. *Cell Death Dis* 2015;6:e2032.
32. Henderson MJ, Richie CT, Airavaara M, Wang Y, Harvey BK. Mesencephalic astrocyte-derived neurotrophic factor (MANF) secretion and cell surface binding are modulated by KDEL receptors. *J Biol Chem* 2013;288:4209–25.
33. Lindholm P, Peranen J, Andressoo JO, et al. MANF is widely expressed in mammalian tissues and differently regulated after ischemic and epileptic insults in rodent brain. *Mol Cell Neurosci* 2008;39:356–71.
34. Apostolou A, Shen Y, Liang Y, Luo J, Fang S. Armet, a UPR-upregulated protein, inhibits cell proliferation and ER stress-induced cell death. *Exp Cell Res* 2008;314:2454–67.
35. Hartley CL, Edwards S, Mullan L, et al. Armet/Manf and Creld2 are components of a specialized ER stress response provoked by inappropriate formation of disulphide bonds: implications for genetic skeletal diseases. *Hum Mol Genet* 2013;22:5262–75.
36. Shen YJ, Sun AM, Wang YH, et al. Upregulation of mesencephalic astrocyte-derived neurotrophic factor in glial cells is associated with ischemia-induced glial activation. *J Neuroinflamm* 2012;9:254.
37. Tadimalla A, Belmont PJ, Thuerlauf DJ, et al. Mesencephalic astrocyte-derived neurotrophic factor is an ischemia-inducible secreted endoplasmic reticulum stress response protein in the heart. *Circ Res* 2008;103:1249–58.
38. Yu YQ, Liu LC, Wang FC, et al. Induction profile of MANF/ARMET by cerebral ischemia and its implication for neuron protection. *J Cerebr Blood F Met* 2010;30:79–91.
39. Kim Y, Lee H, Manson SR, et al. Mesencephalic astrocyte-derived neurotrophic factor as a urine biomarker for endoplasmic reticulum stress-related kidney diseases. *J Am Soc Nephrol* 2016;27:2974–82.
40. Galli E, Harkonen T, Sainio MT, et al. Increased circulating concentrations of mesencephalic astrocyte-derived neurotrophic factor in children with type 1 diabetes. *Sci Rep-uk* 2016;6:29058.



41. Oh-Hashi K, Hirata Y, Kiuchi K. Transcriptional regulation of mouse mesencephalic astrocyte-derived neurotrophic factor in Neuro2a cells. *Cell Mol Biol Lett* 2013;18:398–415.
42. Martindale JJ, Fernandez R, Thuerlauf D, et al. Endoplasmic reticulum stress gene induction and protection from ischemia/reperfusion injury in the hearts of transgenic mice with a tamoxifen-regulated form of ATF6. *Circ Res* 2006;98:1186–93.
43. Glembotski CC, Thuerlauf DJ, Huang C, et al. Mesencephalic astrocyte-derived neurotrophic factor protects the heart from ischemic damage and is selectively secreted upon sarco/endoplasmic reticulum calcium depletion. *J Biol Chem* 2012;287:25893–904.
44. Oh-Hashi K, Tanaka K, Koga H, Hirata Y, Kiuchi K. Intracellular trafficking and secretion of mouse mesencephalic astrocyte-derived neurotrophic factor. *Mol Cell Biochem* 2012;363:35–41.
45. Glembotski CC. Functions for the cardiomyokine, MANF, in cardioprotection, hypertrophy and heart failure. *J Mol Cell Cardiol* 2011;51:512–7.
46. Lindahl M, Danilova T, Palm E, et al. MANF is indispensable for the proliferation and survival of pancreatic beta cells. *Cell Rep* 2014;7:366–75.
47. Voutilainen MH, Back S, Porsti E, et al. Mesencephalic astrocyte-derived neurotrophic factor is neurorestorative in rat model of Parkinson's disease. *J Neurosci* 2009;29:9651–9.
48. Yang S, Huang SS, Gaertig MA, Li XJ, Li SH. Age-dependent decrease in chaperone activity impairs MANF expression, leading to Purkinje cell degeneration in inducible SCA17 mice. *Neuron* 2014;81:349–65.
49. Airavaara M, Chiocco MJ, Howard DB, et al. Widespread cortical expression of MANF by AAV serotype 7: Localization and protection against ischemic brain injury. *Exp Neurol* 2010;225:104–13.
50. Airavaara M, Shen H, Kuo CC, et al. Mesencephalic astrocyte-derived neurotrophic factor reduces ischemic brain injury and promotes behavioral recovery in rats. *J Comp Neurol* 2009;515:116–24.
51. Yang W, Shen YJ, Chen Y, et al. Mesencephalic astrocyte-derived neurotrophic factor prevents neuron loss via inhibiting ischemia-induced apoptosis. *J Neurol Sci* 2014;344:129–38.
52. Neves J, Zhu J, Sousa-Victor P, et al. Immune modulation by MANF promotes tissue repair and regenerative success in the retina. *Science* 2016;353:aaf3646.
53. Palgi M, Lindstrom R, Peranen J, et al. Evidence that DmMANF is an invertebrate neurotrophic factor supporting dopaminergic neurons. *P Natl Acad Sci USA* 2009;106:2429–34.
54. Chen YC, Sundvik M, Rozov S, Priyadarshini M, Panula P. MANF regulates dopaminergic neuron development in larval zebrafish. *Dev Biol* 2012;370:237–49.
55. Yavarna T, Al-Dewik N, Al-Mureikhi M, et al. High diagnostic yield of clinical exome sequencing in Middle Eastern patients with Mendelian disorders. *Hum Genet* 2015;134:967–80.
56. Dooley J, Tian L, Schonefeldt S, et al. Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes. *Nat Genet* 2016;48:519–27.
57. Lindholm P, Voutilainen MH, Lauren J, et al. Novel neurotrophic factor CDNF protects and rescues midbrain dopamine neurons in vivo. *Nature* 2007;448:73–7.
58. Oida Y, Shimazawa M, Imaizumi K, Hara H. Involvement of endoplasmic reticulum stress in the neuronal death induced by transient forebrain ischemia in gerbil. *Neuroscience* 2008;151:111–9.
59. Tajiri S, Oyadomari S, Yano S, et al. Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. *Cell Death Differ* 2004;11:403–15.
60. Szegezdi E, Duffy A, O'Mahoney ME, et al. ER stress contributes to ischemia-induced cardiomyocyte apoptosis. *Biochem Biophys Res Commun* 2006;349:1406–11.
61. Thuerlauf DJ, Marcinko M, Gude N, et al. Activation of the unfolded protein response in infarcted mouse heart and hypoxic cultured cardiac myocytes. *Circ Res* 2006;99:275–82.
62. Zhao H, Liu Y, Cheng L, et al. Mesencephalic astrocyte-derived neurotrophic factor inhibits oxygen-glucose deprivation-induced cell damage and inflammation by suppressing endoplasmic reticulum stress in rat primary astrocytes. *J Mol Neurosci* 2013;51:671–8.
63. Zhu W, Li J, Liu YG, et al. Mesencephalic astrocyte-derived neurotrophic factor attenuates inflammatory responses in lipopolysaccharide-induced neural stem cells by regulating NF- $\kappa$ B and phosphorylation of p38-MAPKs pathways. *Immunopharm Immunot* 2016;38:205–13.
64. Miner JH, Go G, Cunningham J, Patton BL, Jarad G. Transgenic isolation of skeletal muscle and kidney defects in laminin beta2 mutant mice: implications for Pierson syndrome. *Development* 2006;133:967–75.