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Paraoxonase 1 deficiency and hyperhomocysteinemia alter the expression of mouse kidney proteins involved in renal disease ☆

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ABSTRACT

Scope: Hyperhomocysteinemia (HHcy) is associated with kidney disease and leads to atherosclerosis and thrombosis. Paraoxonase 1 (Pon1), a hydrolase that participates in homocysteine (Hcy) metabolism and is carried in the circulation on high-density lipoprotein, has also been linked to kidney disease and atherothrombosis. *Pon1*-knockout mice are susceptible to atherosclerosis and exhibit a kidney-associated phenotype, polyuria or urine dilution. We hypothesize that HHcy and Pon1 deficiency are toxic to kidney function because they impair metabolic pathways important for normal kidney homeostasis.

Methods and results: We examined changes in the mouse kidney proteome induced by *Pon1* gene deletion and dietary HHcy, using 2D IEF/SDS-PAGE gel electrophoresis and MALDI-TOF mass spectrometry. We found that the expression of ten mouse kidney proteins was altered by the $Pon1^{-/-}$ genotype or HHcy. Proteins involved in metabolism of lipid (ApoA-I), protein (Hspd1), carbohydrate (Pdhb, Fbp1-isoform2, Eno1), and energy (Ndufs8, Ldhd) were down-regulated. Proteins involved in lipid transport (Pebp1), oxidative stress response (Prdx2), and cellular detoxification (Glo1) were up-regulated. The kidney proteins altered by HHcy or Pon1 are also altered in renal disease.

Conclusion: Our findings suggest that excess Hcy is toxic because it deregulates the expression of proteins involved in diverse cellular processes–from lipid, protein, carbohydrate, and energy metabolisms to detoxification and antioxidant defenses–that are essential for normal kidney homeostasis. Dysregulation of these processes can account for the involvement of HHcy and reduced Pon1 in kidney disease. Our findings also show that Pon1 plays an important role in maintaining normal kidney homeostasis.

1. Introduction

Paraoxonase 1 (PON1) is expressed in many tissues, including the kidney, liver, lung, brain, and colon (both PON1 mRNA and protein are found in those organs), circulates in the blood attached to high-density lipoproteins (HDL), and is distributed to all tissues both in humans and rodents [1–3]. PON1 has a cardio-protective function in mice [4] and humans [5], which has been linked to its ability to mediate detoxification of oxidized lipids and homocysteine (Hcy)-thiolactone [6]. Reduced PON1 activity is linked to cardiovascular disease [7,8]. In mice, *Pon1* gene deletion impairs urine concentrating ability, which leads to a polyuria phenotype [9].

Pon1 activity is reduced in patients with chronic kidney disease (CKD) [10] and predicts risk of heart attack, stroke, and death [11]. CKD patients also have elevated plasma Hcy [12], an emerging cardiovascular risk factor. Plasma Hcy is negatively correlated with glomerular filtration rate, positively with mortality risk, and accounts for a significant portion of morality in CKD [13,14]. However, mechanisms underlying the toxicity of elevated Hcy and reduced Pon1 in the kidney are not understood [15,16].

Pon1 links HDL and Hcy metabolisms, which may account for their role in kidney disease. Hcy is a negative determinant of HDL and PON1 [17] and reduces *Apoa1* and *Pon1* gene expression [18]. HDL and purified PON1 detoxify Hcy-thiolactone [19] and protect against protein *N*-homocysteinylation [9,20]. *N*-Homocysteinylation causes protein damage and is linked to kidney and cardiovascular diseases (reviewed in [21]). Atherosclerosis-related (*N*-homocysteinylated ApoA-I or *N*-Hcy-ApoA-I) and other *N*-Hcy-proteins [22] increase in hyperhomocysteinemia (HHcy), including CKD patients [12] and kidneys of $Pcft^{-/-}$ mice [21].

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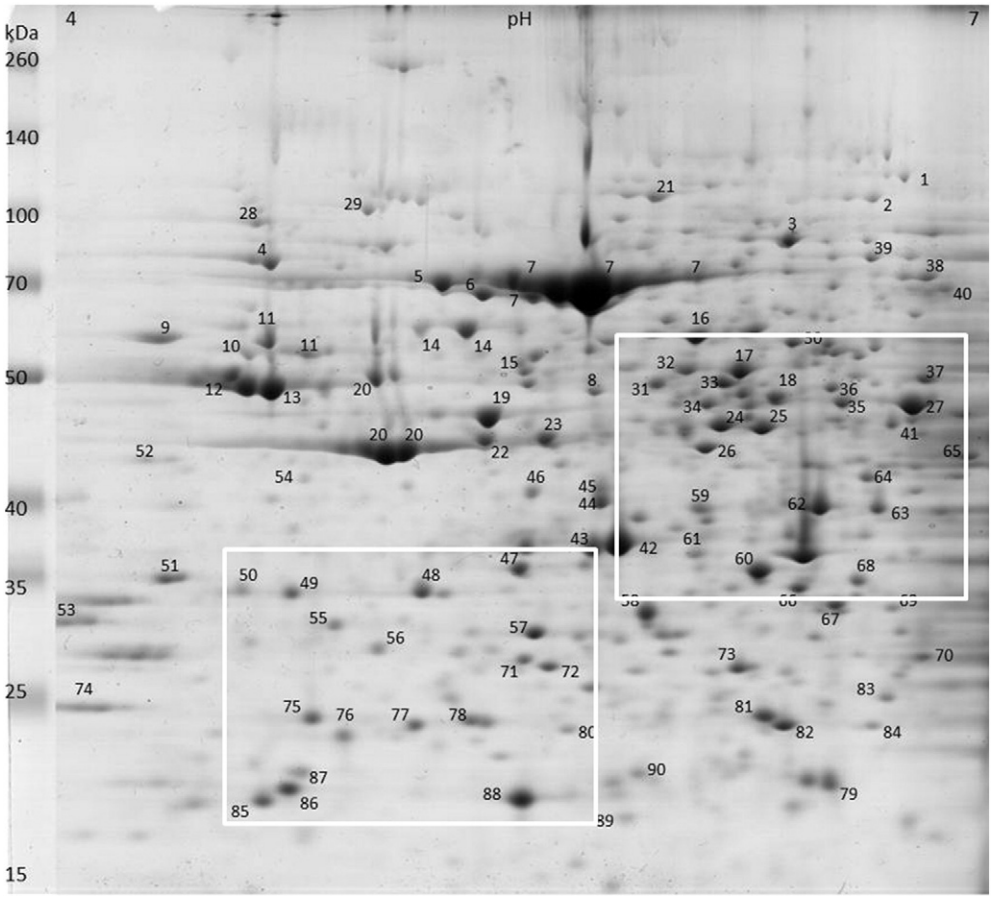

Fig. 1. Representative IEF/SDS-PAGE gel showing the kidney proteome of a wild type C57BL/6J mouse. Left to right: IEF-pH gradient pH 4 → pH 7. Top to bottom: SDS-PAGE; molecular weight markers, 10–260 kDa, shown on the left. Numbers indicate protein spots whose identity was established by mass spectrometry. White rectangles outline areas containing proteins whose expression was affected by the *Pon1* genotype and/or high-Met diet. Identities of the numbered protein spots are described in Supplementary Table S1.

The *Pon1*-null mice are more susceptible to Hcy-thiolactone toxicity than wild type mice and exhibit a polyuria phenotype: produce twice as much urine as their wild-type littermates [9]. Taken together, these findings suggest that Pon1 plays important roles in the kidney.

We hypothesize that HHcy and Pon1 deficiency are toxic because they impair metabolic pathways important for kidney homeostasis. To test this hypothesis, we examined changes in the mouse kidney proteome induced by dietary HHcy in wild type and *Pon1*-null mice.

Table 1
Differentially expressed kidney proteins regulated by $Pon1^{-/-}$ genotype and/or high-Met diet.

Protein description (spot #)	Gene name	Fold change $Pon1^{-/-}$ *vs.* $Pon1^{+/+}$		Fold change std *vs.* 1% Met diet	
		Std diet	1%-Met diet	$Pon1^{+/+}$	$Pon1^{-/-}$
Lipoprotein and lipid metabolism					
Apolipoprotein A-I (#78)	*Apoa1*	−1.47[a]	1.11	−2.27[a]	−1.37[a]
Phosphatidylethanolamine-binding protein 1 (#86)	*Pebp1*	1.32[b]	1.09	1.23	1.02
Amino acid and protein metabolism					
60 kDa heat shock protein, mitochondrial (#14)	*Hspd1*	−1.44[a]	−1.22[b]	−1.22[b]	1.04
Energy metabolism					
NADH dehydrogenase [ubiquinone] iron–sulfur protein 8, mitochondrial (#72)	*Ndufs8*	−1.96[a]	1.07	−2.00[a]	1.05
Probable D-lactate dehydrogenase, mitochondrial (#18)	*Ldhd*	−1.27	1.77[a]	−2.00[a]	1.11
Carbohydrate metabolism					
Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (#47)	*Pdhb*	−1.19[b]	−1.02	−1.44[a]	−1.23[a]
Fructose-1,6-bisphosphatase 1 isoform 2 (#62)	*Fbp1*	−2.00[a]	1.49[b]	−2.27[a]	1.29[c]
Fructose-1,6-bisphosphatase 1 isoform 1 (#63)	*Fbp1*	1.01	−1.01	1.25[c]	1.23
Alpha enolase (#34)	*Eno1*	−2.00[a]	1.17	−1.59[a]	1.48[a]
Oxidative stress response					
Peroxiredoxin 2 (#85)	*Prdx2*	1.88[a]	1.21[a]	1.78[a]	1.14[a]
Methylglyoxal detoxification					
Glyoxalase 1 (#87)	*Glo1*	1.17	1.45[b]	1.08	1.15

Spot # refers to the numbering on the IEF/SDS-PAGE gels in Fig. 1 and Fig. 2.
Significantly different: [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$.