

Chrysan J. Mohammed

Circulating Lactonase Activity but not Protein Level of PON-1 Predicts Adverse Outcomes in Subjects with Chronic Kidney Disease

Supplementary Data

**Table S1.** Clinical characteristics among participants in the CRISIS clinical trial stratified by CKD etiology.

	<b>DM (n = 40)</b>	<b>APKD (n = 16)</b>	<b>Vascular/HTN (n = 85)</b>	<b>GN/Vasculitis (n = 33)</b>	<b>Pyelonephritis (n = 16)</b>	<b>Other (n = 58)</b>	<b>p- value</b>
Age (yr)	68 ± 10	57 ± 15	72 ± 10	58 ± 15	49 ± 20	66 ± 14	<0.0001
Male	26 (65%)	3 (19%)	57 (67%)	21 (64%)	4 (25%)	39 (67%)	0.003
White	40 (100%)	16 (100%)	85 (100%)	33 (100%)	16 (100%)	58 (100%)	-
Hispanic/Latino	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
Height (in)	67 ± 3.5	65 ± 3.3	66 ± 3.6	68 ± 4.0	65 ± 4.0	67 ± 3.1	0.11
Weight (lb)	193 ± 34.2	156 ± 24.2	173 ± 36.5	171 ± 40.6	183 ± 45.6	177 ± 33.6	0.02
BMI (kg/m <sup>2</sup> )	30.0 ± 5.0	26.6 ± 5.0	27.6 ± 5.6	26.7 ± 5.1	30.1 ± 7.7	28 ± 4.4	0.06
Systolic BP (mmHg)	135 ± 22	130 ± 18	141 ± 20	138 ± 19	127 ± 17	136 ± 20	0.09
Diastolic BP (mmHg)	68 ± 12	77 ± 13	72 ± 12	75 ± 10	77 ± 9	76 ± 12	0.004
Urine Protein (mg/dL)	62 ± 133	21 ± 32	50 ± 98	118 ± 168	35 ± 29	44 ± 66	0.01
Creatinine (mg/dL)	2.9 ± 1.6	2.7 ± 1.3	2.6 ± 1.5	2.9 ± 1.5	2.1 ± 0.7	3.0 ± 2.1	0.35
CKD-EPI-eGFR (ml/min per 1.73 m <sup>2</sup> )	27.8 ± 13.1	34 ± 18.5	32 ± 17.0	31 ± 20.0	40 ± 15.0	33 ± 23.2	0.32
<b>Paraoxonase</b>							
Activity (pmol/min/mL)	2032.5 ± 563.9	1941.3 ± 516.0	2159.6 ± 738.0	2313.3 ± 533.2	2127.2 ± 393.6	2190.1 ± 746.8	0.41
Log Activity (pmol/min/mL)	7.6 ± 0.29	7.5 ± 0.29	7.6 ± 0.6	7.7 ± 0.24	7.6 ± 0.19	7.6 ± 0.39	0.63
Median Activity (High)	15 (39%)	5 (31%)	44 (52%)	21 (64%)	9 (56%)	29 (50%)	0.23
Median Activity (Low )	23 (61%)	11 (69%)	41 (48%)	12 (36%)	7 (44%)	29 (50%)	-
Protein (ng/mL)	280.4 ± 294.6	427.1 ± 303.8	436.9 ± 307.5	412.8 ± 213.7	430.9 ± 312.5	480 ± 396.3	0.08
Log Protein (ng/mL)	5.3 ± 0.70	5.8 ± 0.71	5.9 ± 0.65	5.9 ± 0.50	5.9 ± 0.58	6.0 ± 0.59	<0.0001
Median Protein (High)	8 (20%)	8 (50%)	46 (55%)	20 (61%)	8 (50%)	32 (56%)	0.002
Median Protein (low)	32 (80%)	8 (50%)	38 (45%)	13 (39%)	8 (50%)	25 (44%)	-
Adjusted† PON Activity	11.4 ± 5.6	5.5 ± 2.8	6.9 ± 5.4	6.6 ± 2.8	6.2 ± 2.7	6.9 ± 4.4	<0.0001
Log Adjusted† PON Activity	2.3 ± 0.64	1.6 ± 0.61	1.7 ± 0.77	1.8 ± 0.45	1.7 ± 0.54	1.7 ± 0.64	0.0004

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Median Adjusted Activity (High)	31 (82%)	7 (44%)	36 (43%)	17 (52%)	9 (56%)	22 (39%)	0.001
Median Adjusted Activity (Low)	7 (18%)	9 (56%)	48 (57%)	16 (48%)	7 (44%)	35 (61%)	-
<b>CKD Stage</b>							
Mild	0 (0%)	1 (6%)	2 (2%)	2 (6%)	0 (0%)	4 (7%)	
Moderate	15 (38%)	5 (31%)	39 (46%)	12 (36%)	8 (50%)	24 (41%)	
Severe	15 (37%)	6 (38%)	31 (36%)	12 (36%)	7 (44%)	14 (24%)	
ESKD	10 (25%)	4 (25%)	13 (15%)	7 (21%)	1 (6%)	16 (28%)	
<b>Risk Factors/Indications</b>							
Myocardial Infarction	6 (15%)	0 (0%)	25 (29%)	1 (3%)	0 (0%)	9 (15%)	<0.001
Angina	9 (22%)	2 (12%)	25 (29%)	0 (0%)	2 (12%)	11 (19%)	0.001
CVA	1 (3%)	1 (6%)	8 (9%)	1 (3%)	2 (12%)	5 (9%)	0.79
TIA	1 (2%)	1 (6%)	11 (13%)	0	2 (12%)	6 (10%)	0.06
Diabetes Mellitus	36 (98%)	1 (6%)	21 (25%)	2 (6%)	3 (19%)	13 (22%)	<0.0001
Peripheral Vascular disease	9 (23%)	1 (6%)	21 (25%)	1 (3%)	2 (12%)	11 (19%)	0.03
Smoking (current)	4 (10%)	2 (12%)	8 (9%)	7 (21%)	0 (0%)	10 (17%)	0.14
Smoking History	29 (73%)	7 (44%)	65 (76%)	23 (70%)	6 (37%)	41 (71%)	0.02
<b>Medication Use</b>							
ACE	18 (45%)	8 (50%)	28 (33%)	13 (40%)	9 (56%)	20 (34%)	0.40
ARB	15 (37%)	4 (25%)	18 (21%)	12 (36%)	3 (19%)	9 (15%)	0.10
ACE/ARB	29 (72%)	12 (75%)	45 (53%)	23 (70%)	12 (75%)	28 (48%)	0.04
β-Blocker	9 (22%)	6 (37%)	35 (41%)	8 (24%)	4 (25%)	12 (21%)	0.09
Diuretic	26 (65%)	4 (25%)	47 (55%)	14 (42%)	4 (25%)	18 (31%)	0.001
Statin	27 (67%)	6 (27%)	55 (65%)	22 (67%)	3 (19%)	27 (47%)	0.001
Aspirin	24 (60%)	4 (25%)	45 (53%)	3 (9%)	3 (19%)	25 (43%)	<0.0001
<b>Endpoints</b>							
Composite*	28 (70%)	14 (87%)	59 (69%)	24 (73%)	5 (31%)	36 (60%)	0.02
Mortality	23 (57%)	6 (37%)	50 (59%)	14 (42%)	2 (12%)	31 (53%)	0.008
RRT	9 (22%)	10 (62%)	13 (15%)	16 (48%)	3 (19%)	15 (26%)	0.003

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MACE\*\* 4 (10%) 2 (12%) 10 (12%) 0 (0%) 2 (12%) 5 (9%) 0.19

Values are expressed as mean  $\pm$  SD. DM indicates diabetic nephropathy; APKD: adult polycystic kidney disease; vascular/HTN: vascular hypertension; BP: blood pressure; CKD-EC-eGFR: chronic kidney disease-epidemiology collaboration-eGFR; ESKD: end-stage kidney disease; CVA: cerebral vascular accident; TIA: transient ischemic accident; ACE: angiotensin converting enzyme; ARB: angiotensin II receptor blocker. \* Composite endpoint indicates the first occurrence of any of the following events: mortality (cardiovascular or renal death), MACE (myocardial infarction, congestive heart failure, or stroke), and renal replacement therapy. \*\*MACE indicates major adverse cardiovascular event comprising either myocardial infarction, congestive heart failure, or stroke.

**Table S2.** Clinical characteristics among non-CKD healthy controls

	N (%)	Mean $\pm$ SD
Age (yr)		30 $\pm$ 10
Male	15 (45%)	
White	26 (81%)	
Black	1 (3%)	
Asian	3 (9%)	
Other	2 (6%)	
Height (m)		1.7 $\pm$ 1.0
Weight (kg)		80 $\pm$ 19
BMI (kg/m <sup>2</sup> )		28.5 $\pm$ 7.0
Systolic Blood Pressure (mmHg)		117 $\pm$ 10
Diastolic Blood Pressure (mmHg)		74 $\pm$ 7
Creatinine (mg/dL)		0.89 $\pm$ 0.21
CKD- Epidemiology Collaboration-eGFR (ml/min per 1.73 m <sup>2</sup> )		102.7 $\pm$ 16.9
<b>Paraoxonase</b>		
PON Lactonase Activity (pmol/min/mL)		3514.9 $\pm$ 745.1
PON-1 Protein (ng/mL)		682.9 $\pm$ 214.0

Values are expressed as mean  $\pm$  SD.

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**Table S3.** Unadjusted and adjusted 8-year hazard ratio for death at 8 years stratified by quartile values for circulating PON activity, protein adjusted activity levels, and PON-1 protein.

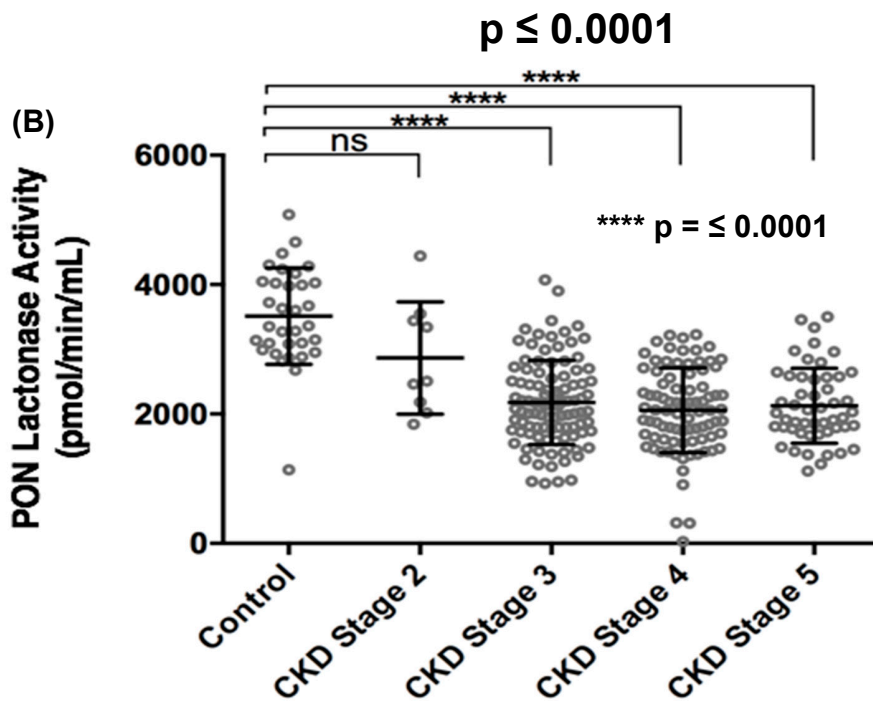
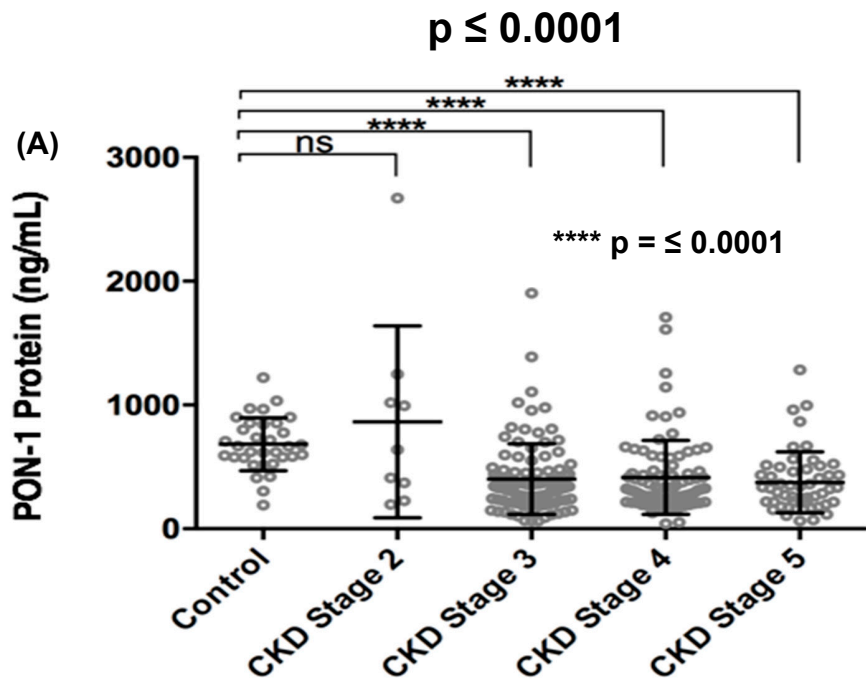
PON Activity (pmol/min/mL)				
	Q 4	Q3	Q2	Q1
<i>Range</i>	≥2576	2073–2576	1732–2073	<1732
Unadjusted HR	1	1.12 (0.64–1.93) <i>p</i> =	1.66 (0.99–2.78) <i>p</i> =	1.76 (1.04–2.97) * <i>p</i> =
Adjusted HR	1	1.01 (0.57–1.78) <i>p</i> =	1.24 (0.74–2.09) <i>p</i> =	1.92 (1.12–3.29) * <i>p</i> =
<b>8-year Death (%)</b>	25/62 = 40.3	26/62 = 41.9	35/61 = 57.4	34/62 = 54.8
Protein Adjusted PON Activity (pmol/min/ng)				
	Q 4	Q3	Q2	Q1
<i>Range</i>	≥8.99	6.22–8.99	4.47–6.22	<4.47
Unadjusted HR	1	1.01 (0.59–1.75) <i>p</i> =	1.24 (0.72–2.12) <i>p</i> =	1.71 (1.03–2.85)
Adjusted HR	1	0.96	0.44	* <i>p</i> =0.04
<b>8-year Death (%)</b>	25/62 = 40.3	27/61 = 44.3	29/61 = 47.5	38/61 = 62.3
PON-1 Protein (ng/mL)				
	Q 4	Q3	Q2	Q1
<i>Range</i>	≥472.35	333.4–472.35	226.85–333.4	<226.85
Unadjusted HR	1	0.69 (0.42–1.14) <i>p</i> =	0.80 (0.49–1.30) <i>p</i> =	0.81 (0.49–1.33) <i>p</i> =
Adjusted HR	1	0.14	0.36	0.40
<b>8-year Death (%)</b>	35/62 = 56.5	0.63 (0.38–1.05) <i>p</i> =	0.72 (0.43–1.18) <i>p</i> =	0.89 (0.54–1.46) <i>p</i> =
		0.08	0.19	0.64
<b>8-year Death (%)</b>	35/62 = 56.5	28/62 = 45.2	29/61 = 47.5	29/62 = 46.8

Model adjusted for traditional risk factors including age, gender, systolic blood pressure, urine protein (log), myocardial infarction, β-blocker, and ACE/ARB. HR indicates hazard ratio; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker. \**p* < 0.05, \*\**p* < 0.01.

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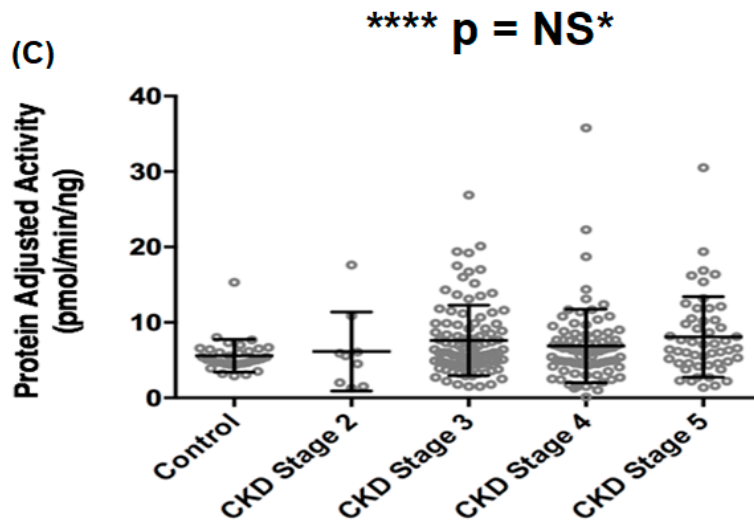
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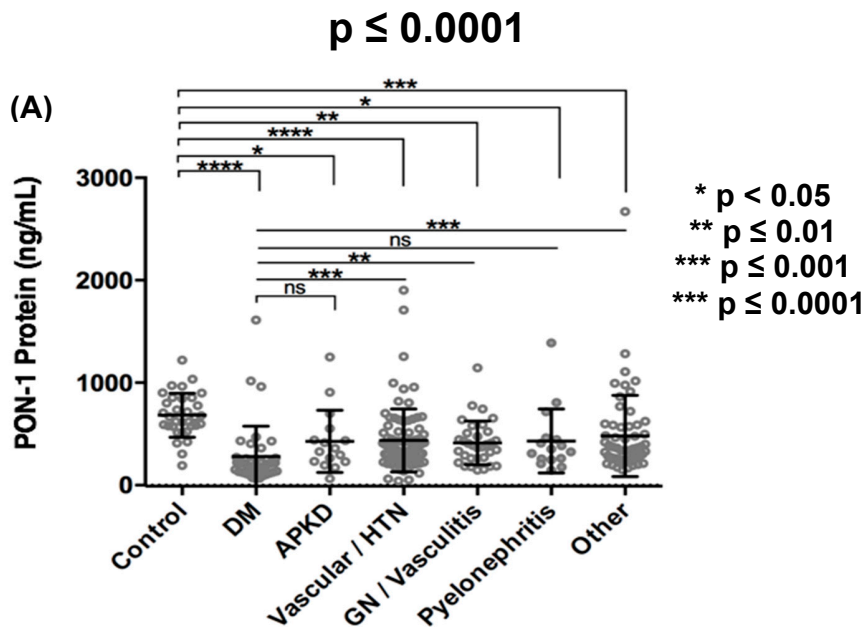
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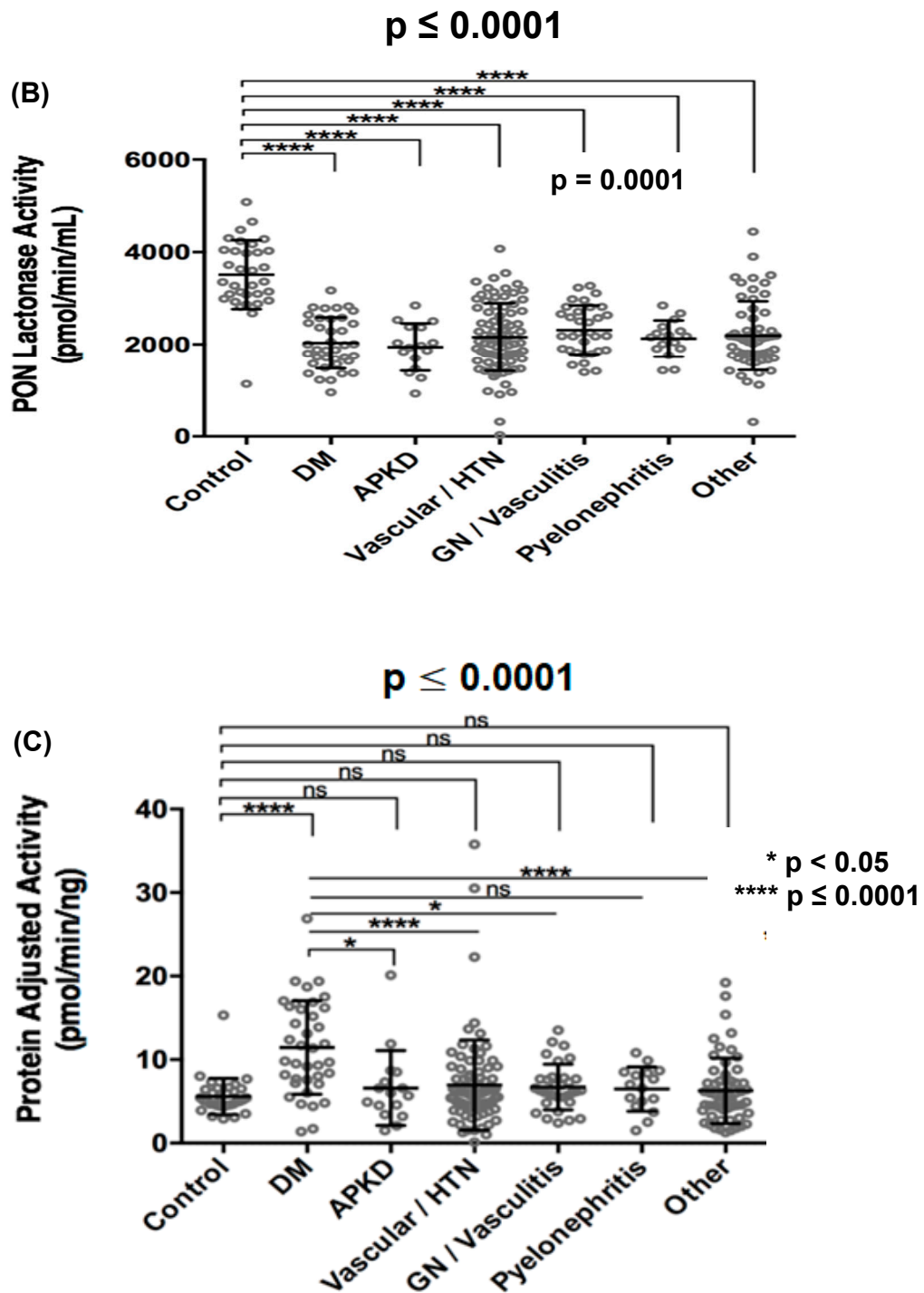
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**Figure 1.** (A) Comparison of circulating PON-1 protein levels across CKD stages. NS indicates not significant. (B) Comparison of circulating PON lactonase activity across CKD stages. NS indicates not significant. (C) Comparison of circulating PON protein adjusted lactonase activity across CKD stages. Protein adjusted lactonase activity is the ratio of PON activity and PON-1 protein. NS indicates not significant.



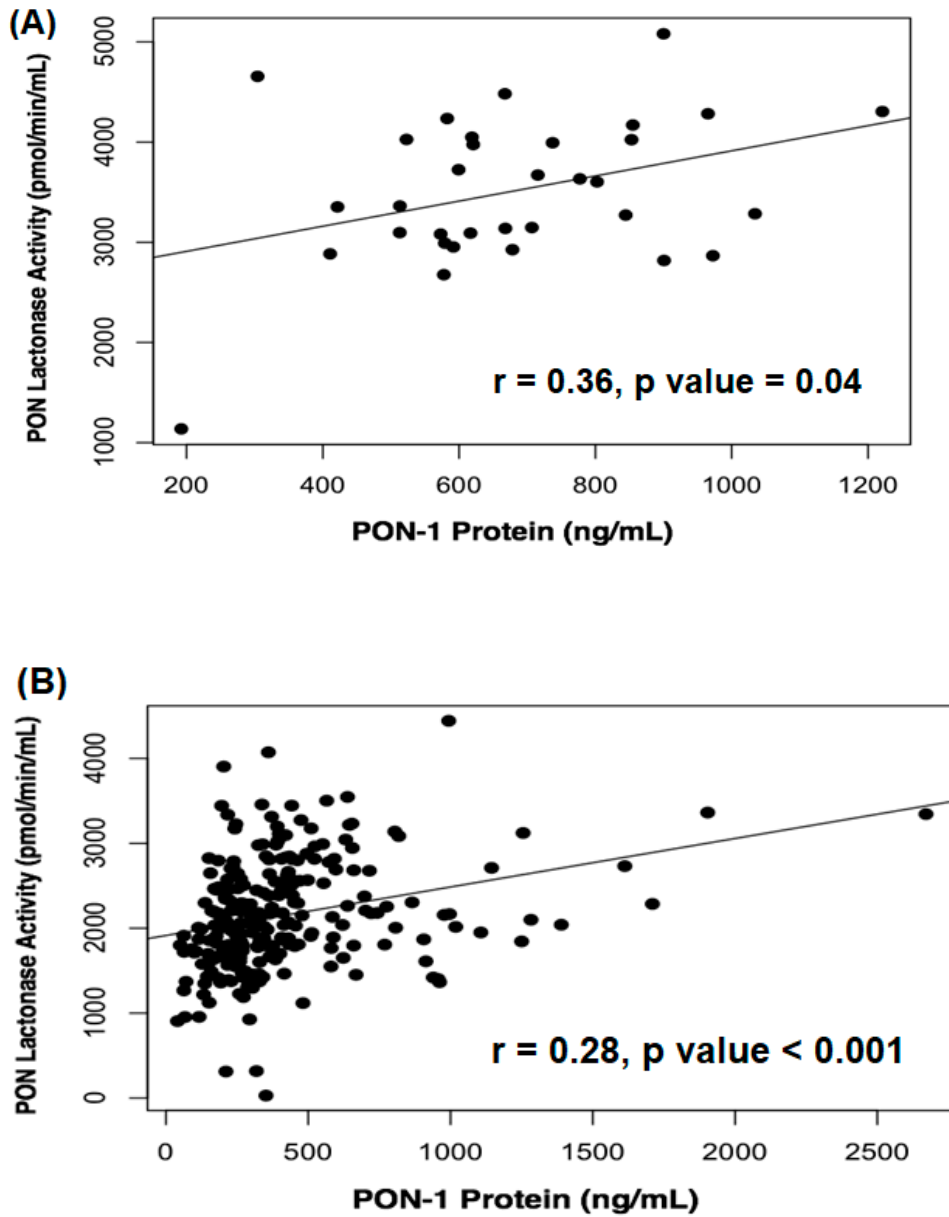


**Figure 2.** (A) Comparison of circulating PON-1 protein levels in CKD etiologies. DM indicates diabetes nephropathy; APKD: adult polycystic kidney disease; vascular/HTN: vascular hypertension; GN/VAS: glomerulonephritis vasculitis; NS: not significant. (B) Comparison of circulating PON lactonase activity in CKD etiologies. DM indicates diabetes nephropathy; APKD: adult polycystic kidney disease; vascular/HTN: vascular hypertension; GN/VAS: glomerulonephritis vasculitis. (C) Comparison of circulating protein adjusted activity in CKD etiologies. DM indicates diabetes nephropathy; APKD: adult polycystic kidney disease; vascular/HTN: vascular hypertension; GN/VAS: glomerulonephritis vasculitis; NS: not significant.

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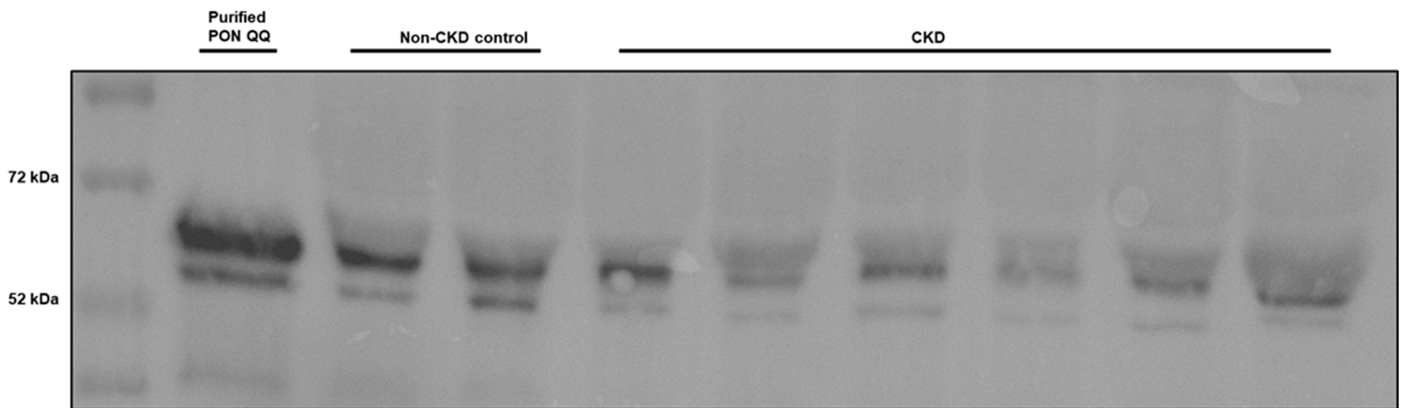
**Figure S3.** (A). Correlation graph showing relationship between PON lactonase activity and PON-1 protein in non-CKD controls. (B) Correlation graph showing relationship between PON lactonase activity and PON-1 protein in patients with chronic kidney disease.



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**Figure S4.** Western blot analysis of PON1 expression in non-CKD control subjects and patients with CKD