3. Tzur S, Rosset S, Shemer R, et al. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. *Hum Genet*. 2010;128:345–350.
4. Gbadegesin RA, Adeyemo A, Webb NJ, et al. HLA-DQA1 and PLCG2 are candidate risk loci for childhood-onset steroid-sensitive nephrotic syndrome. *J Am Soc Nephrol*. 2015;26:1701–1710.
5. Guaragna MS, Lutaif AC, Piveta CS, et al. Two distinct WT1 mutations identified in patients and relatives with isolated nephrotic proteinuria. *Biochem Biophys Res Commun*. 2013;441:371–376.
6. Guaragna MS, Lutaif AC, Piveta CS, et al. NPHS2 mutations account for only 15% of nephrotic syndrome cases. *BMC Med Genet*. 2015;16:88.
7. Guaragna MS, Cleto TL, Souza ML, et al. NPHS1 gene mutations confirm congenital nephrotic syndrome in four Brazilian cases: a novel mutation is described. *Nephrology (Carlton)*. 2016;21:753–757.
8. Feltran LS, Varela P, Silva ED, et al. Targeted next-generation sequencing in brazilian children with nephrotic syndrome submitted to renal transplant. *Transplantation*. 2017;101:2905–2912.

# Identification of Two Forms of Human Plasma Renalase, and Their Association With All-Cause Mortality

J. Chang[1,2,3], X. Guo[1,2], V. Rao[1,2], E.S. Gromisch[3,4,5,6,7], S. Chung[2], H.M. Kluger[1,2,8], C. Cha[2,9], F. Gorelick[1,2,3,10], J. Testani[1,2], R. Safirstein[1,2,3], S. Crowley[1,2,3], A.J. Peixoto[1,2] and G.V. Desir[1,2,3]

[1]Department of Medicine, Yale School of Medicine, New Haven, Connecticut, USA; [2]Department of Medicine, Yale School of Medicine, New Haven, Connecticut, USA; [3]Veterans Affairs Connecticut Health System, University of Connecticut School of Medicine, Farmington, Connecticut, USA; [4]Department of Neurology, University of Connecticut School of Medicine, Farmington, Connecticut, USA; [5]Mandell Center for Multiple Sclerosis, Mount Sinai Rehabilitation Hospital, Trinity Health of New England, Hartford, Connecticut, USA; [6]Department of Rehabilitative Medicine, Frank H. Netter MD School of Medicine, Quinnipiac University, North Haven, Connecticut, USA; [7]Department of Medical Sciences, Frank H. Netter MD School of Medicine, Quinnipiac University, North Haven, Connecticut, USA; [8]Section of Medical Oncology, Yale School of Medicine, New Haven, Connecticut, USA; [9]Department of Surgery, Yale School of Medicine, New Haven, Connecticut, USA; and [10]Department of Cell Biology, University of Connecticut School of Medicine, Farmington, Connecticut, USA

**Correspondence**: Gary Desir, Section of Nephrology, Department of Medicine, Yale School of Medicine, P.O. Box 208029, New Haven, Connecticut 06520-8029, USA. E-mail: gary.desir@yale.edu

**Received 9 August 2019; revised 15 November 2019; accepted 6 December 2019; published online 16 December 2019**

*Kidney Int Rep* (2020) **5,** 362–368; https://doi.org/10.1016/j.ekir.2019.12.002


Renalase (RNLS) is a recently discovered flavoprotein produced and secreted by a variety of tissues including the kidneys.[1–3] Inside the cell, RNLS as a nicotinamide adenine dinucleotide (NADH) oxidase regulates energy metabolism.[4] Outside the cell, RNLS acts as a potent pro-survival signal when it binds to its cell membrane receptor, the plasma membrane calcium adenosine triphosphatase isoform PMCA4, and activates a variety of intracellular signaling pathways including the protein kinase B (AKT), extracellular-signal-regulated kinase (ERK), and signal transducer and activator of transcription 3 (STAT3) pathways.[5–7] Administration of RNLS minimizes injury in *in vivo* models of myocardial infarction,[8] ischemic tubular necrosis,[9] and acute pancreatitis.[S1] Conversely, RNLS deficiency in RNLS knockout mice exacerbates cisplatin-mediated acute and chronic renal injury, which is reversed by administration of RNLS.[1,9,S2] Dysregulated RNLS signaling appears to promote survival of malignant cells from several tumor types by augmenting expression of growth-related genes. Increased tissue RNLS expression in patients with pancreatic cancer and melanoma was associated with increased mortality.[S3,S4]

No standardized and validated method of measuring RNLS concentrations in human plasma currently exists. In cohorts of subjects with normal renal function, a commercially available enzyme-linke immunosorbent assay (ELISA) using a monoclonal antibody yields widely variable results, with the concentrations ranging from as low as 1.18 ± 0.44 ug/ml (mean ± SD) to as high as 39.80 ± 14.63 (mean ± SD), a 33-fold difference.[S5–S10]

We hypothesized that the variations in plasma concentrations measured by Western blot and commercially available ELISA may be due to the

existence of different forms of RNLS and the different capacities of detection antibodies to recognize them. We have developed a sensitive and efficient method that detects 2 distinct forms of RNLS in human plasma—free and bound RNLS. In a prospective study, we determined the relationship between plasma RNLS and renal function, and examined the association of plasma RNLS and all-cause mortality.

## RESULTS

### Measurement of Plasma RNLS by Western Blot

We estimated plasma RNLS concentrations in 15 subjects with normal renal function (defined as estimated glomerular filtration rate [eGFR] > 60 cc/min per 1.73 $m^2$), by probing polyacrylamide gels run under non-denaturing and non-reduced conditions with m42-RNLS, and obtained a value of 19.98 ± 5.09 μg/ml (n = 15). Representative Western blots are shown in Figure 1a, and b. Note that under these native conditions, probing for RNLS with 2 different RNLS-specific antibodies (m42-RNLS and AF5350) revealed plasma RNLS migrating as a single broad band with an apparent molecular weight of ∼120 kDa, instead of the expected monomeric molecular weight of 37 kDa. Size exclusion chromatography of human plasma from adult males with normal renal function showed that RNLS circulates in many forms, all of which are larger than the RNLS monomer (Figure 1c). These findings suggest that plasma RNLS is present as oligomers or in complexes with other proteins.

### ELISA Development

#### Choice of Capture and Detection Antibodies

We tested 2 monoclonal antibodies (mAbs; m28-RNLS and m42-RNLS) and 3 commercially available polyclonal anti-RNLS antibodies (Supplementary Methods and Supplementary Table S1) in 11 different combinations to identify the pair that performed best for measuring recombinant human (rh)RNLS. As shown in Supplementary Table S2, m42-RNLS and Ab178700 (Abcam) performed best as capture antibodies. For detection of rhRNLS, Ab312291 (Abcam, Cambridge, UK) and 1C11E8 (Novus, Centennial, CO) performed well. We selected m42-RNLS for capture because it worked best in native Western blot and recognized the native conformation of the RNLS complex. The polyclonal Ab312291 was selected for detection because it was raised against a peptide that mediates RNLS binding to PMCA4b.[S11]

#### Choice of Pretreatment Methods

Although we could detect RNLS in the native human plasma with ELISA, employing m42-RNLS and Ab31291 as capture and detection antibodies, respectively, the measured concentrations were ∼100-fold lower than those obtained by Western blot also run under the native conditions. We hypothesized that there were 2 distinct forms of RNLS in the native human plasma. In one form of RNLS, the binding site for PMCA4b is exposed and thus freely accessible by the detection antibody Ab312291, which was raised against a RNLS peptide that mediates binding to PMCA4b. We labeled this form *free* RNLS (F-RNLS),

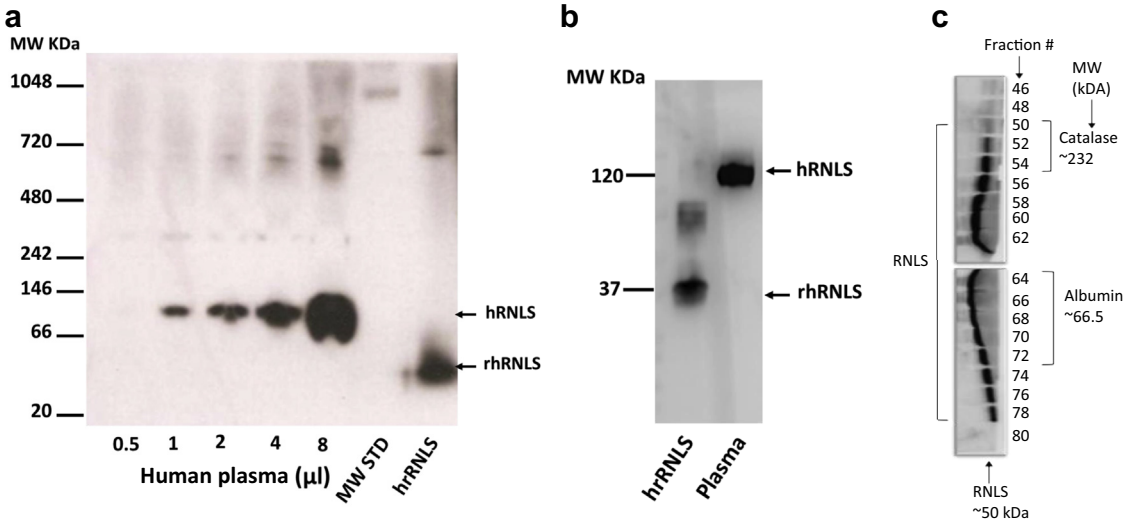


**Figure 1.** Apparent molecular weight (MW) of human plasma renalase (RNLS). (a) RNLS detected by Western blot of human plasma run under native conditions: proteins separated by native gel electrophoresis and probed for RNLS using the goat polyclonal AF5350; recombinant human renalase (rhRNLS) = 100 ng recombinant human RNLS; amount of human plasma (0.5–8 μl) indicated under each lane; arrows indicate human RNLS (hRNLS) protein. (b) Proteins separated by native gel electrophoresis and probed for RNLS using the rabbit monoclonal m42-RNLS; arrows indicate hRNLS protein. (c) Distinct forms of plasma RNLS separated by gel permeation chromatography: human plasma separated by gel permeation chromatography using Sepharose CL-6B; fractions are further separated by sodium dodecylsulfate gel electrophoresis and probed for hRNLS using rabbit monoclonal m28-RNLS; arrow at bottom indicates hRNLS. STD, standard.

the form that is measured by our ELISA in the untreated native plasma. In the other form of RNLS, the binding site for PMCA4b is hidden, rendering it inaccessible and thus unmeasurable by the detection antibody Ab31229; we designated the latter form *bound* RNLS (B-RNLS). Total RNLS (T-RNLS) is the sum of bound and free RNLS. We then tested different methods to expose the hidden binding site in the native plasma and found that acidifying the plasma for 10 minutes with either HCl or citric acid followed by neutralization with $Na_2HPO_4$ unveiled the previousy hidden binding site for the detection antibody Ab312291 and yielded plasma RNLS concentrations comparable in range to those obtained by Western blot (Supplementary Methods and Figure 2). Each plasma sample was thus assayed twice, without acid-pretreatment to measure F-RNLS, and with acid-pretreatment to measure T-RNLS.

### *Choice of Reference Standard*

We have found that in the absence of reducing agents, rhRNLS forms multimers even after short storage periods. This property of rhRNLS could introduce significant errors in establishing standard curves, making rhRNLS unsuitable for use as a reference standard. To improve the reproducibility of the ELISA, we synthesized 3 novel reference standards or RNLS peptide calibrators (RPCs) (Supplementary Methods and Supplementary Figure S1A). RPC 1 and 2 each consisted of the m42-RNLS peptide antigen (VLEALKNYI: C terminus of human RNLS), a linking peptide 15 or 11 amino acids long, and the m28-RNLS peptide antigen (CIRFVSIDNKKRNIESSEIGP; aa 220-240 of human RNLS). RPC 3 was formed by joining the same m42-RNLS peptide antigen to a truncated m28 antigen, which notably contains the antigen for the goat polyclonal Ab31291.

We then compared standard curves generated using rhRNLS, rhRNLS with acid pretreatment (as above), RPC1, RPC2, and RPC3. rhRNLS contains 12 cysteines and requires the addition of reducing agents if stored for more than a few days. Freshly made rhRNLS performed well as a reference standard, as did RPC1, RPC2, and RPC3. We found an excellent correlation between RPC1 and RPC2, and between RPC2 and rhRNLS ($R^2 = 0.96$ and $R^2 = 0.94$ respectively). We obtained the best correlation ($R^2 = 0.97$) between RPC3 and acid-treated rhRNLS (Supplementary Figure S1B). Due to its ease of synthesis, its superior stability (because of lack of cysteine), and its excellent correlation with rhRNLS, RPC3 was chosen as the reference standard.

## Renalase and GFR

Renalase concentrations were measured using our ELISA in 267 patients recruited from outpatient primary care, renal and dialysis clinics at the Veterans Affairs Connecticut Healthcare System. The cohort had the mean age of 69 years and was all male patients, with 79% white and 18% black (Table 1). The eGFRs in the cohort spanned the entire spectrum of renal function. The plasma concentration of T-RNLS was 18.8 ± 8.5 μg/ml (Supplementary Figure S2). The plasma concentration of F-RNLS was 0.66 ± 0.16 μg/ml and represented 4.0% ± 2.5 % of T-RNLS. In a subgroup of patients with normal renal function (defined as eGFR > 60 cc/min per 1.73 $m^2$), the plasma concentration of T-RNLS was

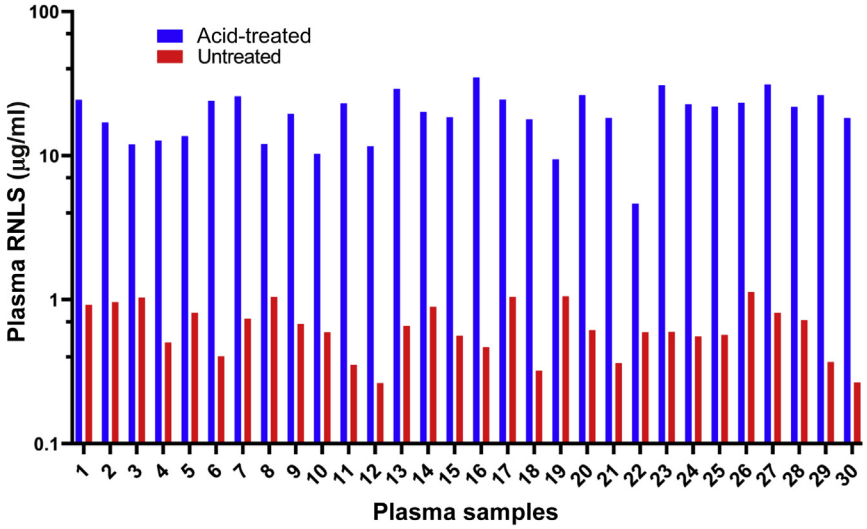


**Figure 2.** Free and bound renalase (RNLS) levels in human plasma. Human plasma samples (20 μl) were incubated with 1M citric acid (10 μl) for 10 minutes at room temperature. The samples were then neutralized by the addition of 1M disodium phosphate (20 μl). RNLS concentrations are determined by enzyme-linked immunosorbent assay using m42-RNLS for capture, AB31291 for detection, and the RNLS peptide RPC3 as the calibrator. Human RNLS concentration in untreated and acid-treated samples are compared for each sample.

**Table 1.** Baseline demographics (N = 267)

| Characteristics | Value |
|---|---|
| Age, yr | 69 ± 12 |
| Race (white/black/other) | 211/48/8 (79/18/3) |
| Body mass index, kg/m$^2$ | 29 ± 5.7 |
| Systolic/diastolic blood pressure, mm Hg | 137/67 ± 22/13 |
| eGFR stage (eGFR in cc/min per m$^2$) | |
| I (>90) | 28 (10) |
| II (60–89) | 91 (34) |
| III (30–59) | 71 (27) |
| IV (15–29) | 46 (17) |
| V (<15) | 31 (12) |
| Diabetes mellitus | 102 (38) |
| Hypertension | 215 (81) |
| Myocardial infarction | 41 (15) |
| Congestive heart failure | 38 (14) |

eGFR, estimated glomerular filtration rate.
Values are n (%), unless otherwise indicated.

20.39 ± 7.70 μg/ml. This value closely matched that obtained by Western blot (19.89 ± 6.23 μg/ml).

In bivariate analysis using Spearmns's rho (ρ), log10 T-RNLS correlated positively with eGFR (r = 0.27, $P <$ 0.001) and negatively with eGFR stages (ρ = –0.27, $P <$ 0.001; Figure 3). By contrast, the log10 F-RNLS fraction correlated negatively with eGFR (r = –0.21, $P = 0.001$) and positively with eGFR stages (ρ = 0.24, $P < 0.001$). No significant association was found between GFR (or eGFR stages) and log10 F-RNLS.

Compared to eGFR stage I, eGFR stage V was significantly associated with T-RNLS (b = –0.20, $P <$ 0.001) in the multivariate analysis after adjusting for age, race, body mass index, blood pressure, comorbidities, and antihypertensive medications, whereas eGFR stages II, III, and IV were not significant (Table 2). Similarly, compared to eGFR stage I, eGFR stage V was significantly associated with the F-RNLS fraction in the multivariate analysis (b = 0.14, $P = 0.027$), as was eGFR stage IV (b = 0.13, $P = .026$; Table 3).

## Renalase and Mortality

Among 262 subjects included in mortality analysis, 20 patients (8.4%) were deceased at 1 year, 75 patients (28.6%) at 5 years, and 131 patients (50.0%) at 10 years. For 1-year mortality, the log10 F-RNLS fraction was higher in deceased individuals (0.65 ± 0.27) than in living individuals (0.52 ± 0.25; $P = .030$). No significant difference was found in log10 F-RNLS ($P =$ 0.18) or log10 T-RNLS ($P = 0.22$) between deceased and living individuals at 1 year (Table 4). For 5-year mortality, the log10 F-RNLS fraction was also higher in deceased individuals (0.59 ± 0.26) compared to living individuals (0.51 ± 0.25; $P = .014$). Moreover, log10 F-RNLS was higher in deceased (2.80 ± 0.20) than in living individuals (2.74 ± 0.24; $P = .048$). There was no significant difference in log10 T-RNLS between the deceased and living individuals ($P = .384$). None of the RNLS variables was a significant predictor of mortality at 10 years.

In multivariable logistic regression, the log10 F-RNLS fraction was an independent predictor of both 1-year mortality (odds ratio [95% confidence interval]:

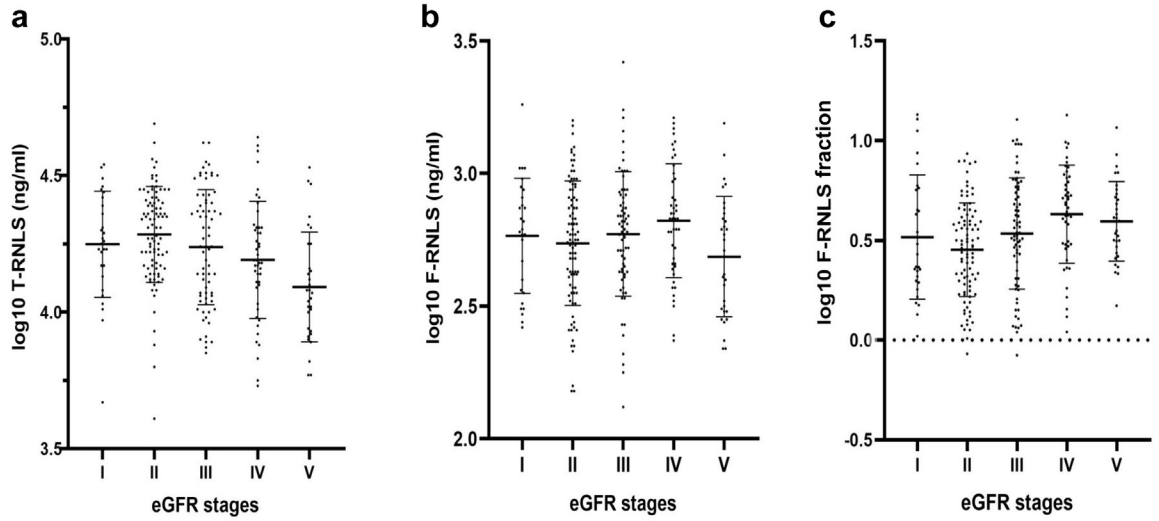


**Figure 3.** Estimated glomerular filtration rate (eGFR) stages versus renalase (RNLS). eGFR stages versus total (T)-RNLS, free (F)-RNLS, and F-RNLS fraction. The associations between eGFR stages and untransformed RNLS values could not be examined because the values did not satisfy the normality requirement. Therefore, the RNLS values were transformed using a log transformation (log10). (a) Log10 T-RNLS was negatively associated with advancing eGFR stages (ρ = –0.27, $P < 0.001$). The association remained significant after adjusting for covariates (β = –4.90, $P < 0.001$). (b) Log10 F-RNLS was not associated with eGFR stages. (c) Log10 F-RNLS fraction was positively associated with advancing eGFR stages (ρ = 0.24, $P < 0.001$). The association remained significant after adjusting for covariates (β = 0.24, $P < 0.001$). Stages of eGFR: stage I = eGFR >90; stage II = eGFR 60–89; stage III = eGFR 30–59; stage IV = eGFR 15–29; and stage V = eGFR <15 or dialysis (eGFR in cc/min per 1.73 m$^2$). RNLS values are shown as mean ± 95% confidence intervals.

**Table 2.** Multivariate analyses for the association between estimated glomerular filtration rate stages and log10 total-renalase (N = 265)

| Variable | B | SE B | β | *P* |
|---|---|---|---|---|
| Constant | 4.55 | 0.08 | – | – |
| Body mass index | –0.01 | 0.00 | –0.21 | <0.001 |
| Calcium channel blocker | –0.05 | 0.02 | –0.12 | 0.037 |
| Stage I vs. stage II | 0.02 | 0.04 | 0.05 | 0.588 |
| Stage I vs. stage III | –0.03 | 0.04 | –0.06 | 0.559 |
| Stage I vs. stage IV | –0.07 | 0.05 | –0.12 | 0.162 |
| Stage I vs. stage V | –0.20 | 0.05 | –0.32 | <0.001 |

B, intercept; SE, standard error.
$R^2 = 0.16$.

**Table 3.** Multivariate analyses for the association between estimated glomerular filtration rate stages and log10 free-renalase fraction (N = 265)

| Variable | B | SE B | β | *P* |
|---|---|---|---|---|
| Constant | 0.21 | 0.15 | – | – |
| Body mass index | 0.01 | 0.00 | 0.26 | <0.001 |
| Mean blood pressure | –0.00 | 0.00 | –0.10 | 0.097 |
| Vasodilators | 0.10 | 0.06 | 0.10 | 0.089 |
| Stage I vs. stage II | –0.03 | 0.05 | –0.06 | 0.571 |
| Stage I vs. stage III | 0.04 | 0.06 | 0.06 | 0.510 |
| Stage I vs. stage IV | 0.13 | 0.06 | 0.20 | 0.026 |
| Stage I vs. stage V | 0.14 | 0.07 | 0.18 | 0.027 |

B, intercept; SE, standard error.
$R^2 = 0.15$.

9.2 [1.2, 74]) and 5-year mortality (odds ratio [95% confidence interval]: 3.8 [1.1, 13.1]) even after adjusting for age, race, mean blood pressure, body mass index, and Charlson comorbidity score (Table 5). Log10 F-RNLS also independently predicted death at 5 years (odds ratio [95% confidence interval: 4.6 [1.1, 18.5]). Not surprisingly, both age and the Charlson comorbidity index were independent predictors of mortality.

## DISCUSSION

In this study, we identified 2 immunologically distinct forms of RNLS in human plasma—T-RNLS and F-RNLS—using a new ELISA method. We believe that T-RNLS constitutes the whole of renalase in human plasma and that F-RNLS represents its biologically active form. With the use of the same capture antibody m42, the RNLS concentration by ELISA in the acid-treated plasma (i.e., T-RNLS) is nearly identical to the RNLS concentration by Western blot run under native conditions. Only F-RNLS, however, is recognized in the untreated native plasma by the detection antibody Ab31291. Because the detection antibody Ab31291 targets a region of RNLS (aa 224–234, human RNLS) critical for binding to its cell membrane receptor PMCA4b and for activating intracellular signaling, F-RNLS may represent a portion of T-RNLS that has its PMCA4b-binding site exposed, and is thus able to effect intracellular signaling. In contrast, most of T-RNLS in the native human plasma is unrecognizable by the detection antibody Ab3129, presumably because its PMCA4b-binding site is concealed. Unable to bind to PMCA4B and activate cell signaling, most of T-RNLS remains biologically inactive. F-RNLS makes up only about 4% of T-RNLS. Thus, only a small portion of plasma RNLS exists in an active form.

The notion that RNLS circulates in plasma in both active and inactive forms is not a new one. We previously reported that in response to an intravenous bolus of epinephrine, the amine oxidase activity of plasma RNLS in rats increased dramatically in less than a minute, and that this effect persisted long after the catecholamine bolus stopped.[S12] Because the stimulation of RNLS activity was seen immediately after the catecholamine infusion, it was unlikely to have resulted from synthesis of new RNLS or even secretion of preformed RNLS. In fact, an RNLS level by Western blot was reduced at 1 and 5 minutes after epinephrine infusion. Possibly, RNLS that became activated underwent a conformational change that compromised its detection by the polyclonal anti-renalase antibody used to probe the Western blot.

Our clinical data lend support to the idea that F-RNLS is the biologically active form. In our cohort of outpatients with variable renal function, both the F-RNLS and F-RNLS fractions were independent predictors of mortality. In contrast, T-RNLS showed no such association with mortality. Our finding is in line with results of 2 previous studies using a commercial ELISA that also have shown RNLS to be an independent predictor of mortality.[S13,S14]

The causal links between RNLS and mortality need further investigation. RNLS promotes survival of malignant cells from several tumor types, and a higher tissue expression of RNLS in pancreatic cancer and melanoma was a predictor of death. Thus, the heightened risk of mortality associated with the F-RNLS and F-RNLS fractions may stem from cancer development and cancer death. RNLS may be a marker of chronic inflammation, a precursor of multiple lethal diseases. Chronic inflammation is a strong predictor of a poor outcome in chronic kidney disease, and deteriorating renal function is accompanied by heightened inflammatory responses and elevated inflammatory markers. Interestingly, in our cohort of 267 veterans with variable renal function, deteriorating renal function was associated with a significant increase in F-RNLS fraction, which, in turn, was associated with higher mortality. Oxidative stress also may be the causal link

**Table 4.** Multivariate analyses for 1-year and 5-year mortality with log10 free-renalase fraction as predictor (N = 262)

| | 1-year mortality[a] | | | | 5-year mortality[b] | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | B | SE B | Odds ratio (95% CI) | *P* | B | SE B | Odds ratio (95% CI) | *P* |
| Constant | −11.23 | 4.67 | – | – | −8.27 | 2.25 | – | – |
| Age | 0.14 | 0.05 | 1.15 (1.05, 1.26) | 0.002 | 0.08 | 0.02 | 1.08 (1.04, 1.12) | <0.001 |
| Race | −0.65 | 0.75 | 0.52 (0.12, 2.27) | 0.387 | 0.14 | 0.41 | 1.15 (0.52, 2.55) | 0.730 |
| Mean blood pressure | −0.00 | 0.02 | 1.00 (0.95, 1.04) | 0.830 | 0.00 | 0.01 | 1.01 (0.98, 1.03) | 0.735 |
| Body mass index | −0.11 | 0.06 | 0.90 (0.79, 1.02) | 0.090 | −0.03 | 0.03 | 0.97 (0.91, 1.03) | 0.284 |
| Charlson comorbidity | 0.38 | 0.11 | 1.46 (1.17, 1.82) | <.001 | 0.43 | 0.08 | 1.54 (1.30, 1.81) | <0.001 |
| Log10 free-renalase fraction | 2.22 | 1.06 | 9.22 (1.16, 73.59) | 0.036 | 1.32 | 0.65 | 3.75 (1.06, 13.32) | 0.041 |

B, intercept; CI, confidence interval; SE, standard error.
[a]One-year mortality: $R^2 = 0.15$ (Cox and Snell), 0.36 (Nagelkerke), model $\chi^2(6) = 42.35$, $P < .001$.
[b]Five-year mortality: $R^2 = 0.25$ (Cox and Snell), 0.35 (Nagelkerke), model $\chi^2(6) = 74.18$, $P < .001$.

between RNLS and mortality.[S15] In a recent study of hemodialysis patients, plasma RNLS levels using a commercial ELISA correlated well with advanced oxidation protein products, a well-established marker of oxidative stress, and the accumulation of which has been associated with increased cardiovascular events in chronic kidney disease.[S16] Finally, high F-RNLS and F-RNLS fractions may be indirect markers of increased sympathetic activity, a driver of mortality in many diseases including chronic kidney disease and congestive heart failure.

Renalase in human plasma appears to circulate in several different forms. On Western blots run under native conditions, RNLS from human plasma migrates as a broad band with an apparent molecular weight of ~120 kDa, a value that far exceeds its predicted homomeric molecular weight of 37 kDa. In contrast, RNLS in tissues and cells migrates at 37 kDa, even under native conditions. Incidentally, under denatured and reduced conditions, plasma RNLS migrates at ~37–50 kDa.[1] Gel permeation chromatography confirmed the presence of multiple distinct forms of RNLS in human plasma, ranging in size from ~200 to 90 kDa. When sequential column fractions (~250–90 kDa) are collected and the proteins separated by sodium dodecylsulfate polyacrylamide gel electrophoresis, rhRNLS migrates at ~37–50 kDa, well below the size of the RNLS peaks observed after fractionation of human plasma. These results suggest that RNLS in human plasma circulates either as homomultimers or bound to carrier proteins. We cannot completely exclude the possibility that a small proportion of plasma RNLS is monomeric and is below the detection limit of our current Western blotting methods. Whether F-RNLS is a monomer, a multimer, or bound to a carrier protein remains unanswered.

**Table 5.** Multivariate analyses for mortality with log10 free renalase as predictor (N = 262)

| | 5-year mortality | | | |
|---|---|---|---|---|
| Variable | B | SE B | Odds ratio (95% CI) | *P* |
| Constant | −11.81 | 3.03 | – | – |
| Age | 0.08 | 0.02 | 1.08 (1.04, 1.12) | <0.001 |
| Race | 0.10 | 0.41 | 1.11 (0.50, 2.48) | 0.799 |
| Mean blood pressure | 0.00 | 0.01 | 1.00 (0.98, 1.03) | 0.833 |
| Body mass index | −0.03 | 0.03 | 0.97 (0.92, 1.03) | 0.383 |
| Charlson comorbidity | 0.45 | 0.09 | 1.58 (1.33, 1.86) | <0.001 |
| Log10 free-renalase | 1.52 | 0.71 | 4.59 (1.14, 18.51) | 0.032 |

B, intercept; CI, confidence interval; SE, standard error.
$R^2 = 0.25$ (Cox and Snell), 0.36 (Nagelkerke), model $\chi^2(6) = 74.68$, $P < .001$.

The study had a few limitations.[S17] First, our cohort included only male patients. The significant associations of renalase concentrations with eGFR and mortality require confirmation in female patients. Second, the cause-specific mortality was not examined as an outcome in our study because of unavailability of data on the specific causes of death. We plan to examine this question in another cohort with information on the specific causes of death.

In conclusion, using a new ELISA that targets its biologically active region, we have shown that RNLS in plasma exists in 2 distinct conformations. The majority of renalase has its biologically active region hidden, to be unveiled only by acid pretreatment. Only in the minority of renalase is its active region exposed in untreated plasma and thus detectable by our ELISA. We hypothesize that F-RNLS represents the active conformation that is responsible for cyto-protection in tissue injury and for stimulation of cell growth in tumors. In support of this possibility, F-RNLS and F-RNLS fractions were independently predictive of mortality. Future studies should focus on understanding the factors that regulate the balance between T-RNLS and F-RNLS and confirming the association and testing the potential causal links between plasma RNLS and mortality.

## DISCLOSURE

GVD is a named inventor on several issued patents related to the discovery and therapeutic use of renalase. Renalase is licensed to Bessor Pharma, and GVD holds an equity

position in Bessor and its subsidiary Personal Therapeutics. All the other authors declared no competing interests.

## ACKNOWLEDGMENTS

This work was supported in part by VA Connecticut (RS and GVD); VA Merit Award (FG and GVD); National Institutes of Health grants RC1DK086465, RC1DK086402, and R01DK081037 (GVD), and RO1CA216846 (GVD and HMK); National Cancer Institute Research Grant CA-16359; National Cancer Institute Grant K24CA172123 (HMK); and the Lampman Surgical Award from the Yale University Department of Surgery (CC).

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

**Figure S1.** ELISA development.

**Figure S2.** eGFR stages versus plasma renalase.

**Table S1.** Listing of anti-RNLS antibodies evaluated.

**Table S2.** Selection of antibody pairs suitable for use in ELISA.

**STROBE Statement.**

**Supplementary References.**

**Supplementary Methods.**

## REFERENCES

1. Xu J, Li G, Wang P, et al. Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. *J Clin Invest*. 2005;115:1275–1280.
2. Desir VG. The author replies: Does renalase degrade catecholamines? *Kidney Int*. 2011;79:2.
3. Weinman EJ, Biswas R, Steplock D, et al. Increased renal dopamine and the acute renal adaptation to a high phosphate diet. *Am J Physiol Renal Physiol*. 2011;300:F1123–1129.
4. Farzaneh-Far R, Desir GV, Na B, et al. A functional polymorphism in renalase (Glu37Asp) is associated with cardiac hypertrophy, dysfunction, and ischemia: data from the heart and soul study. *PLoS One*. 2010;5:e13496.
5. Guo X, Wang L, Velazquez H, et al. Renalase: its role as a cytokine, and an update on its association with type 1 diabetes and ischemic stroke. *Curr Opin Nephrol Hypertens*. 2014;23:513–518.
6. Wang L, Velazquez H, Chang J, et al. Identification of a receptor for extracellular renalase. *PLoS One*. 2015;10:e0122932.
7. Giordano FJ, Wang Y, Desir GV. A remote role for renalase. *EBioMedicine*. 2016;9:27–28.
8. Wu Y, Xu J, Velazquez H, et al. Renalase deficiency aggravates ischemic myocardial damage. *Kidney Int*. 2011;79:853–860.
9. Lee HT, Kim JY, Kim M, et al. Renalase protects against ischemic AKI. *J Am Soc Nephrol*. 2013;24:445–455.

# DNAJB9 Is Not Transcriptionally Upregulated in the Glomerulus in Fibrillary Glomerulonephritis



Rupali S. Avasare[1], Bridget A. Robinson[2], Jonathan Nelson[1], Randy Woltjer[3], Victoria Krajbich[3], Vy Nguyen[3], Daphne Garcia[3], Naly Setthavongsack[3], Chris Kizzar[3], Phillip W. Raess[3], Susan B. Gurley[1], Kelly D. Smith[4] and Nicole K. Andeen[3]

[1]Department of Medicine, Division of Nephrology and Hypertension, Oregon Health & Science University, Portland, Oregon, USA; [2]Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Portland, Oregon, USA; [3]Department of Pathology, Oregon Health and Science University, Portland, Oregon, USA; and [4]Department of Pathology, University of Washington, Seattle, Washington, USA

**Correspondence**: Nicole K. Andeen, 3181 SW Sam Jackson Park Road, Mail code: L113, Portland, Oregon 97239, USA. E-mail: andeen@ohsu.edu

**Received 14 November 2019; revised 2 December 2019; accepted 9 December 2019; published online 16 December 2019**

*Kidney Int Rep* (2020) **5,** 368–372; https://doi.org/10.1016/j.ekir.2019.12.004



Fibrillary glomerulonephritis (FGN) is an immune-complex–mediated GN with high rates of progression to end-stage kidney disease.[1,2] DnaJ homolog subfamily B member 9 (DNAJB9) is a sensitive and specific marker of FGN in kidney biopsies.[3–5] DNAJB9 is a heat-shock protein in the endoplasmic reticulum and is involved in the endoplasmic reticulum stress/unfolded response (UPR) pathway; it also binds aggregation-prone peptides.[6–9,S1–S3] Upregulation of other UPR proteins in FGN was not detected in mass spectrometry–based studies,[3] suggesting that the glomerular accumulation of DNAJB9 is not due to local upregulation of the UPR in glomeruli. In addition, serum levels of DNAJB9 were modestly increased in