Elevated Galectin-3 Precedes the Development of CKD

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ABSTRACT
Galectin-3, a profibrotic mediator, is linked to the development of renal fibrosis in animal models and inversely correlates with GFR in humans, but whether galectin-3 predicts incident kidney disease is unknown. Here, we assessed renal outcomes for 2450 Framingham Offspring participants who attended examination 6 (1995–1998) and had follow-up data at examination 8 (2005–2008). Renal outcomes of interest included rapid decline in renal function (≥3 ml/min per 1.73 m² per year decline in estimated GFR [eGFR]), CKD (eGFR < 60 ml/min per 1.73 m²), and albuminuria (albumin-to-creatinine ratio ≥17 mg/g in men or ≥25 mg/g in women). We used multivariable logistic regression models to evaluate associations between galectin-3 with incident renal outcomes at examination 8. During a mean follow-up of 10.1 years, GFR declined rapidly in 241 (9.2%) participants, incident CKD developed in 277 (11.3%), and albuminuria developed in 194 (10.1%). Higher plasma levels of galectin-3 were associated with rapid decline in eGFR (per 1-SD log-galectin-3; adjusted odds ratio [OR], 1.49; 95% confidence interval [CI], 1.28 to 1.73) and a higher risk of incident CKD (OR, 1.47; 95% CI, 1.27 to 1.71), but not with the risk of incident albuminuria. The addition of galectin-3 to clinical predictors improved the C-statistic (0.837–0.845; P = 0.02) but did not reach predefined thresholds for clinically significant improvements to risk prediction based on reclassification indices. In conclusion, elevated levels of plasma galectin-3 are associated with increased risks of rapid GFR decline and of incident CKD in the community, which calls for further study in higher-risk groups.


CKD is a major worldwide public health problem¹–⁶ that may result in progressive deterioration in kidney function,⁷ substantial morbidity,⁸–¹⁰ and increased mortality from both cardiovascular¹¹ and noncardiovascular causes.¹² Because of the lack of early clinical signs or symptoms and the poor sensitivity of currently available biomarkers (serum creatinine and urinary protein), CKD is typically detected at an advanced stage. However, when detected early, kidney function decline may be slowed or even reversed and these secondary complications averted.¹³¹⁵ Hence, there is a pressing clinical need for novel biomarkers that identify at-risk individuals at the earliest possible stage.

Galectin-3 is a β-galactoside–binding lectin that has emerged as a key regulator of inflammation and fibrosis.¹⁶ Galectin-3 is strongly linked to the development of organ fibrosis in rodent models: Galectin-3 knockout mice are resistant to the development of fibrosis, whereas infusion of recombinant galectin-3 induces TGF-β–dependent tissue fibroblast proliferation and collagen deposition.¹⁷¹⁸

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Clinical Correlates of Galectin-3

We determined Pearson correlation coefficients because galectin-3 appeared strongly associated with several CKD risk factors at baseline. We observed a modest inverse correlation between galectin-3 and baseline eGFR (Pearson partial correlation \( r = -0.15; P \leq 0.0001 \) (Table 2), which was substantially attenuated after adjustment for age and sex. The unadjusted correlation between galectin-3 and baseline albuminuria was weak (\( r = 0.07; P = 0.001 \)) and attenuated after adjustment for age and sex (\( P = 0.07 \)) (Table 2).

Galectin-3 and Rapid Decline in Renal Function

Of 2613 participants assessed, 241 (9.2%) experienced a rapid decline in renal function during follow-up. Galectin-3 was associated with rapid decline in eGFR in age- and sex-adjusted (odds ratio [OR], 1.38 per 1-SD increase in log-galectin-3; 95% confidence interval [CI], 1.21 to 1.58; \( P < 0.0001 \)) and multi-variable-adjusted (OR, 1.49; 95% CI, 1.27 to 1.73; \( P < 0.0001 \)) analyses (Table 3).

Similarly, the risk for rapid loss of renal function increased by galectin-3 quartile in both age- and sex-adjusted (OR for Q4 versus Q1 2.61; 95% CI, 1.70 to 4.01; \( P < 0.0001 \)) and multi-variable-adjusted (OR for Q4 versus Q1, 2.93; 95% CI, 1.84 to 4.67; \( P < 0.0001 \)) analyses (Figure 1).

Galectin-3 and Incident CKD

Incident CKD developed in 277 (11.3%) participants during follow-up. There was an increased risk of incident CKD per 1-SD increase in log-galectin-3 in age- and sex-adjusted analyses (OR, 1.56; 95% CI, 1.35 to 1.80; \( P < 0.0001 \)) (Table 3). Results

Table 1. Baseline characteristics by sex-specific quartile of galectin-3 concentration in the Framingham Heart Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectin-3 cut-points (men/women) (ng/ml)</td>
<td>3.9/5.0</td>
<td>10.9/11.8</td>
<td>12.7/13.9</td>
<td>15/16.3</td>
<td>—</td>
</tr>
<tr>
<td>Patients (men/women), n, (n/n)</td>
<td>615 (279/336)</td>
<td>616(296/320)</td>
<td>608(288/320)</td>
<td>611(279/322)</td>
<td>—</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>53±8</td>
<td>56±9</td>
<td>58±8</td>
<td>60±9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Women (%)</td>
<td>54.6</td>
<td>51.9</td>
<td>52.6</td>
<td>54.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>24.9</td>
<td>31.8</td>
<td>38.2</td>
<td>45.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Hypertension treatment (%)</td>
<td>13.5</td>
<td>19.8</td>
<td>24.5</td>
<td>32.9</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123±18</td>
<td>125±17</td>
<td>128±18</td>
<td>129±18</td>
<td>0.06</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.6±4.4</td>
<td>27.9±5.3</td>
<td>28.1±5.1</td>
<td>28.5±5.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>4.9</td>
<td>7.5</td>
<td>5.9</td>
<td>10.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>13.5</td>
<td>13.5</td>
<td>16.2</td>
<td>13.9</td>
<td>0.009</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53.6±16</td>
<td>51.9±16</td>
<td>51.6±16</td>
<td>49.5±16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UACR (IQR) (mg/g)</td>
<td>5.9(2.8, 12.6)</td>
<td>5.0(2.5, 10.8)</td>
<td>5.8(2.3, 12.0)</td>
<td>7.0(2.7, 15.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>Baseline eGFR (ml/min per 1.73 m²)</td>
<td>92.8±14</td>
<td>90.4±16</td>
<td>88.5±16</td>
<td>86±15</td>
<td>0.01</td>
</tr>
<tr>
<td>eGFR* at follow-up (ml/min per 1.73 m²)</td>
<td>84.6±14</td>
<td>81.3±15</td>
<td>77.7±15</td>
<td>73.3±16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CKD at follow-up (%)</td>
<td>4.7</td>
<td>7.8</td>
<td>12.2</td>
<td>20.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Galectin-3 (IQR) (ng/ml)</td>
<td>10.1(9.1, 10.7)</td>
<td>12.3(11.7, 12.8)</td>
<td>14.45(13.9, 15.1)</td>
<td>17.3(16.5, 19.2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Quartiles were derived from untransformed data. Data are presented as means ± SD for normally distributed continuous variables, median and IQR for continuous variables that are not normally distributed, or percentage for categorical data. \( P \) value for trend across quartiles. CKD was defined as eGFR < 60 ml/min per 1.73 m² using the CKD-Epidemiology Collaboration equation. SBP, systolic BP; UACR, urinary albumin-to-creatinine ratio; IQR, interquartile range.

* Trend test \( P \) value adjusted for age and sex.
were similar after multivariable adjustment (OR, 1.47; 95% CI, 1.27 to 1.71; \( P<0.0001 \)), and additional adjustment for aldosterone and homocysteine did not materially affect the results (OR, 1.38; 95% CI, 1.18 to 1.61; \( P<0.0001 \)). Similarly, additional adjustment for body mass index, which is correlated with galectin-3, minimally altered the results (OR, 1.46; 95% CI, 1.26 to 1.70; \( P<0.0001 \)). The association remained significant after adjustment for cystatin C at interim examination 7 (OR, 1.20; 95% CI, 1.02 to 1.42; \( P=0.03 \)).

The risk of incident CKD also increased by baseline quartile of galectin-3 (age- and sex-adjusted OR for Q4 versus Q1, 2.98; 95% CI, 1.91 to 4.64; \( P<0.0001 \)) (Figure 1), and there was minimal attenuation with multivariable adjustment (OR for Q4 versus Q1, 2.5; 95% CI, 2.59 to 3.98; \( P<0.0001 \)) or multivariable plus multimarker (homocysteine and aldosterone) adjustment (OR for Q4 versus Q1, 2.32; 95% CI, 1.42 to 3.77; \( P=0.0007 \)).

**Table 2.** Correlation coefficients for log galectin-3 in the Framingham Heart Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>SBP</th>
<th>HDL</th>
<th>Glucose</th>
<th>eGFR</th>
<th>Log UACR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>0.11</td>
<td>-0.02</td>
<td>0.04</td>
<td>-0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>( P ) value</td>
<td>&lt;0.0001</td>
<td>0.4</td>
<td>0.04</td>
<td>&lt;0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>0.02</td>
<td>-0.10</td>
<td>0.01</td>
<td>-0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>( P ) value</td>
<td>0.3</td>
<td>&lt;0.0001</td>
<td>0.6</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Age-, sex-, and eGFR-adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>0.02</td>
<td>-0.10</td>
<td>0.02</td>
<td>–</td>
<td>0.04</td>
</tr>
<tr>
<td>( P ) value</td>
<td>0.3</td>
<td>&lt;0.0001</td>
<td>0.4</td>
<td>–</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Data presented as Pearson correlation coefficient above with associated \( P \) value below for each variable at baseline. SBP, systolic BP; UACR, urinary albumin-to-creatinine ratio.

**Table 3.** Odds of kidney disease per 1-SD increase of log galectin-3 (ng/ml) in the Framingham Heart Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident CKD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.56 (1.35 to 1.80)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.47 (1.27 to 1.71)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.38 (1.18 to 1.61)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>+ homocysteine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ aldosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid eGFR decline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.38 (1.21 to 1.58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.49 (1.27 to 1.73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Incident albuminuria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.05 (0.89 to 1.23)</td>
<td>0.6</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.04 (0.88 to 1.23)</td>
<td>0.6</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>0.99 (0.83 to 1.18)</td>
<td>0.9</td>
</tr>
<tr>
<td>+ homocysteine,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ aldosterone, and BNP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Galectin-3 and Incident Albuminuria**

There were 194 (10.1%) new cases of albuminuria at follow-up. There was no evidence of an increased risk of incident albuminuria in age- and sex-adjusted \( OR, 1.05; 95\% CI, 0.89 to 1.23; \( P=0.6 \)) or multivariable-adjusted \( OR, 1.04; 95\% CI, 0.88 to 1.23; \( P=0.6 \)) (Table 3) analyses per 1-SD increase in log-galectin-3, nor did the risk of incident albuminuria increase by galectin-3 quartile \( (P>0.1 \) for all comparisons) (Figure 1).

**C-Statistic and Reclassification Analyses**

The C statistic for the basic CKD clinical prediction model, comprising age, sex, diabetes, hypertension, dipstick proteinuria, and baseline eGFR, was 0.837. The addition of galectin-3 improved the C statistic to 0.845 \( (P=0.02) \).

Galectin-3 improved categorical reclassification of CKD by 3.5% when added to the clinical model \( (95\% CI, 0.4 \) to 6.7), which did not reach the prespecified threshold for clinical significance of 5%. The category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI) for the addition of galectin-3 in predicting CKD similarly did not reach the predefined threshold for clinical significance \( (NRI, 0.35 [95\% CI, 0.23 to 0.48]; IDI, 0.01 [95\% CI, 0.005 to 0.02]) \).

**DISCUSSION**

**Principal Findings**

The findings of the present study are four-fold. First, higher levels of galectin-3 were associated with an increased risk of incident CKD in the general population. These findings were robust in continuous and categorical analyses after adjustment for known clinical predictors of CKD, as well as circulating biomarkers known to enhance CKD prediction. Second, galectin-3 showed similarly strong associations with rapid loss of renal function over 10 years of follow-up. Third, there was no association between galectin-3 and albuminuria in any analysis. Finally, although improvements in the C-statistic and reclassification indices for the addition of galectin-3 were statistically significant, they did not exceed our predefined thresholds for clinical significance. Taken together, these data suggest that galectin-3 may identify individuals at risk for the development of CKD many years before clinical onset and, more important, suggest an important role for fibrosis early in the pathogenesis of CKD.

**In the Context of the Current Literature**

Higher galectin-3 levels have been linked to an increased all-cause mortality risk in the general population.\(^31\) Furthermore, galectin-3 predicts both acute and chronic heart failure and is a predictor of prognosis in that setting.\(^28,32–35\) Higher plasma galectin-3 levels were cross-sectionally associated with lower GFR in 133 patients with chronic systolic heart failure.\(^26\) Our study extends upon these observations by
demonstrating robust associations with *de novo* CKD and rapid loss of renal function in prospective analyses of apparently healthy members of the general population.

These findings extend the literature on novel biomarkers of CKD risk. We have previously shown that both serum aldosterone and homocysteine are associated with incident CKD independent of traditional risk factors and result in a small but significant reclassification improvement. Similar observations were made for a higher aldosterone-to-renin ratio and incident CKD in an Asian community-based cohort. Our study extends the literature in this regard by identifying a novel biomarker of *de novo* kidney disease that is independent of both traditional risk factors and these novel predictors of renal risk. Furthermore, unlike aldosterone and homocysteine, galectin-3 has a very modest baseline correlation with GFR ($r = -0.05; P = 0.02$).

**Potential Mechanisms**

Galectin-3 is a functionally diverse 32- to 35-kDa member of the galectin family of $\beta$-galactoside–binding lectins, a group functionally characterized by the presence of a carbohydrate recognition domain. It is expressed both intracellularly, where it regulates proliferation and apoptosis via carbohydrate-independent mechanisms, and on the cell surface and extracellular space, where it modulates cell-cell interactions (including cell adhesion, activation, and chemotraction) and regulates cell growth, differentiation, and inflammation via its carbohydrate-binding functions. In the kidney, galectin-3 plays a complex role that is context-dependent. In development, it appears to promote normal nephrogenesis, being strongly expressed in the ureteric bud and its derivatives, with subsequently lower expression levels in the mature tubule.

Galectin-3 plays a pro-resolution role in inflammation and repair. It is intensely upregulated in response to ischemic and nephrotoxic AKI, preventing chronic tubular injury by limiting apoptosis, enhancing matrix remodeling and attenuating fibrosis. The accelerated development of diabetic nephropathy in galectin-3 $^{-/-}$ mice is further evidence of renoprotective properties. However, when tissue injury is persistent or repetitive, galectin-3 may modulate the transition to chronic inflammation and fibrosis. It is a potent activator of fibroblasts in a range of tissues, including the kidney, liver, gut, and myocardium. Kidney fibrosis in human transplant recipients also depends on galectin-3. As such, galectin-3 may have proresolution or profibrotic effects along the continuum from acute inflammation to chronic inflammation and ultimately fibrosis.

Although galectin-3 is believed to play a causal role in the development of fibrosis of certain human organs, such as heart and liver, we cannot address whether a similar causal relationship might explain our results. Other explanations are possible. For example, the significant unadjusted inverse correlation between galectin-3 and baseline GFR suggests that galectin-3 might be handled by the kidney and thus be a surrogate for kidney function. We believe this is unlikely to be the case...
sole explanation for our findings for three reasons. First, the
clearance of galectin-3 appears to be primarily hepatic in or-
igin on the basis of prior studies.53 Second, participants in our
study were free of CKD at the time of assay, so patients with
elevations in galectin-3 due to CKD were excluded. Finally,
the correlation between galectin-3 and baseline GFR was
not significant after adjustment for age and sex. Another pos-
sibility is that elevations in galectin-3 might occur as a re-
spone to renal injury but have no direct involvement in
CKD progression.

Implications
As well as enhancing our ability to identify individuals at risk
for CKD, our study gives insight into potential underlying
biologic mechanisms. For example, it suggests that subclinical
tubulointerstitial fibrosis may be important in the early stages
of CKD, whereas the lack of association with albuminuria
argues against glomerular injury or foot process effacement as a
mechanism. Furthermore, because elevations in galectin-3
were identified years before overt kidney disease, they may
offer a window of opportunity to initiate early, preventive
treatment aimed at preventing disease occurrence. This is
important because efforts to attenuate the pro-fibrotic effects of
galactin-3 in a mouse model of AKI have shown promise.56 A
nontoxic pectin derivative found in citrus fruit, modified cit-
rus pectin, decreased circulating levels of galectin-3 by binding
the carbohydrate recognition domain and resulted in signifi-
cant attenuation of renal fibrosis and inflammation in mouse
models.57 Further research is necessary to determine whether
galactin-3 is along the causal pathway for the development of
CKD and whether targeting galectin-3 can reduce CKD
development.

Finally, although statistically significant, improvements in
the C-statistic and reclassification indices did not exceed
our predefined thresholds for clinical significance. Nonethe-
less, the weak signal observed in this healthy, community-
based cohort raises the possibility of a stronger effect in
higher-risk groups, which is an interesting avenue for future
study.

Strengths and Limitations
The strengths of our study are our well characterized sample,
prospective cohort design, and an adequate number of CKD
and albuminuria events. Some limitations also require men-
tion. Cystatin C was not available at the baseline examination,
and although we adjusted for its effects at an interim time point,
this may have introduced a survival bias. Nonetheless, results
were consistent with the primary analyses. We do not have a
gold standard measure of GFR because this is not feasible in a
large epidemiologic study. The large number of missing
urinary albumin measures at both baseline and follow-up
reduced the sample size for that analysis. The lack of repeated
measures of galectin-3 over time is also a limitation. Finally, the
sample was elderly and white, limiting generalizability to other
groups.

Conclusions
Higher circulating galectin-3 levels are associated with in-
creased risk of incident CKD and rapid loss of renal function
over time in the community. Our findings suggest that galectin-
3 may detect kidney injury years before the clinical onset of
CKD, potentially affording an opportunity to institute early,
targeted treatment aimed at disease prevention.

CONCISE METHODS

Participants
The design and methods of the Framingham Offspring Study are
described elsewhere.58 Each examination comprises a standardized
medical history, routine questionnaires, physical examination, an-
thropometry, and blood testing. The present study includes partic-
ipants assessed during the sixth examination cycle of the Offspring
Study (1995–1998), at which time galectin-3 was assayed, who also
returned for follow-up at examination 8. All participants provided
written informed consent, and the institutional review boards of the
Boston University Medical Center approved the study.

Of 3448 eligible participants, 5 were excluded for having extreme
galactin-3 measures (>5 log-SDs above or below the log-transformed
mean). Of the remaining 3443 participants, 2450 were included in the
incident CKD analysis after 993 exclusions (lack of follow-up, n=699;
prevalent CKD, n=163; missing baseline creatinine, n=12; missing
follow-up creatinine, n=99; and missing covariates, n=20). There
were 2613 participants in the analysis for rapid decline in eGFR after
830 exclusions (lack of follow-up, n=699; missing baseline creatinine,
n=12; missing follow-up creatinine, n=99; and missing covariates,
n=20). Finally, there were 1919 participants in the incident albumin-
uria analysis after 1524 exclusions (lack of follow-up, n=577; preva-
lent albuminuria, n=339; missing baseline urinary albumin, n=502;
missing follow-up urinary albumin, n=104; and missing covariates,
n=2). Participants who did not attend follow-up tended to be older
(63 versus 57 years), male, and in poorer health (more likely to smoke
or to have diabetes, hypertension, proteinuria, and/or a lower mean
eGFR).

Outcome Definitions
Rapid decline in GFR was defined as the dichotomous outcome of
eGFR loss of ≥3 ml/min per 1.73 m² per year based on two measures
(baseline and follow-up eGFR) using the CKD-Epidemiology Col-
laboration equation.59 This outcome has previously been linked to
cardiovascular disease and all-cause mortality risk in people with
and without CKD.50,61

CKD was defined as an eGFR < 60 ml/min per 1.73 m². Serum
creatinine was measured using the modified Jaffé method and cali-
brated as previously described.62

Albuminuria was defined as a urinary albumin-to-creatinine ratio
≥25 mg/g in women and ≥17 mg/g in men.63 Spot urine samples
collected at the baseline examination (1995–1998) were stored
at −20°C and then transitioned to −80°C. The urinary albumin
concentration was measured using immunoturbidimetry (www.roche.
com), and urinary creatinine levels were measured using the Jaffé
Plasma Galectin-3 Measurement
Blood samples were collected after an overnight fast and immediately centrifuged and stored at −80°C until assayed. Plasma concentrations of galectin-3 were measured using an ELISA (BG Medicine, Waltham, MA). The lower detection limit was 1.32 ng/ml with an upper detection limit of 96.6 ng/ml. The within-run and total precision are reported between 2.1%–5.7% and 4.2%–12.0%, respectively.

Covariate Assessment
Participants underwent blood testing and were assessed for CKD risk factors. HDL cholesterol and blood glucose were measured on morning blood samples while fasting. Diabetes was defined as fasting blood glucose level of ≥126 mg/dl (7 mMol/L) or use of medication for the treatment of diabetes. Systolic and diastolic BP measurements were taken as the mean of two physician readings using a mercury sphygmomanometer. Hypertension was defined as a systolic BP ≥140 mmHg or a diastolic BP ≥90 mmHg or self-reported use of medication for hypertension. Body mass index was defined as an individual’s weight in kilograms divided by height in meters squared. Current smoking status was defined by self-report. Plasma brain natriuretic peptide was measured with high-sensitivity immunoradiometric assays (Shionogi, Japan; coefficient of variation [CV], 12.2%). Serum aldosterone was measured by radioimmunoassay (Quest Diagnostics; CV, 3.8%–6.0%). Plasma total homocysteine was measured by HPLC with fluorometric detection (CV, 9%). Brain natriuretic peptide, aldosterone, and homocysteine were assayed at baseline examination 6. Serum cystatin C was measured using particle-enhanced immunonephelometry (Dade Behring BN 100; CV, 2.4%). Cystatin C was measured only at interim examination 7.

Statistical Analyses
Because of sex differences in galectin-3 distribution, sex-specific quartiles of galectin-3 were used to present baseline descriptive data. Galectin-3 was log-transformed for all subsequent analyses. Baseline clinical characteristics were summarized by quartiles of log-galectin-3, and age-adjusted trends in means across quartiles were compared by ANOVA. We used sex-standardized log-galectin-3 for all correlation and regression analyses. The correlation of galectin-3 with CKD risk factors was examined using age- and sex-adjusted and age-, sex-, and eGFR-adjusted Pearson partial correlation coefficients. Multivariable logistic regression was used to assess relations of galectin-3 to the three outcomes: incident CKD, rapid decline in kidney function, and albuminuria. In these analyses, galectin-3 was modeled both as a continuous variable (odds of outcome per 1-SD increase of log plasma galectin-3) and a categorical variable (odds of renal outcomes by quartiles of log plasma galectin-3). Models were adjusted for (1) age and sex and (2) CKD covariates: age, sex, diabetes, hypertension, dipstick proteinuria, and baseline eGFR. The multivariable model for incident albuminuria was adjusted for age, sex, diabetes, hypertension, body mass index, smoking, HDL cholesterol, and baseline log urinary albumin-to-creatinine ratio. Covariates for these models are derived from prior work. Participants with baseline CKD or albuminuria were excluded from the respective incident analyses. No participants were excluded from the rapid decline in kidney function analysis, and the multivariable model applied the same covariates as for the incident CKD analysis.

In a secondary analysis, we adjusted for biomarkers previously shown to enhance risk prediction of kidney disease beyond clinical factors: serum homocysteine and aldosterone both for incident CKD and aldosterone, brain natriuretic peptide, and homocysteine for incident albuminuria. We also additionally adjusted for cystatin C in a secondary analysis.

To assess the ability of galectin-3 to improve CKD prediction beyond clinical measures, we compared C-statistics for the addition of galectin-3 to a CKD clinical prediction model comprising age, sex, diabetes, hypertension, dipstick proteinuria, and baseline eGFR. We also estimated the category-based and category-free NRI metrics for the addition of galectin-3 to fully adjusted models. We defined cut-points for risk groups for the category-based NRI using tertiles of predicted risk from the model (<3%, 3%–6%, >6%). We also estimated the IDI, which may be interpreted as a continuous version of the NRI with probability differences used instead of categories.

A type I error threshold of 0.05 was used to indicate statistical significance. All statistical analyses were performed using SAS software, version 9.2 (http://www.sas.com/).

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DISCLOSURES
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