Growth Differentiation Factor–15 and Risk of CKD Progression

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ABSTRACT

Growth differentiation factor–15 (GDF-15) is a member of the TGF-β cytokine superfamily that is widely expressed and may be induced in response to tissue injury. Elevations in GDF-15 may identify a novel pathway involved in loss of kidney function among patients with CKD. Among participants in the Clinical Phenotyping and Resource Biobank (C-PROBE) study and the Seattle Kidney Study (SKS), we tested whether kidney tissue expression of GDF15 mRNA correlates with circulating levels of GDF-15 and whether elevations in circulating GDF-15 are associated with decline in kidney function. In matching samples of 24 patients with CKD from the C-PROBE study, circulating GDF-15 levels significantly correlated with intrarenal GDF15 transcript levels (r=0.54, P=0.01). Among the 224 C-PROBE and 297 SKS participants, 72 (32.1%) and 94 (32.0%) patients, respectively, reached a composite end point of 30% decline in eGFR or progression to ESRD over a median of 1.8 and 2.0 years of follow up, respectively. In multivariable models, after adjusting for potential confounders, every doubling of GDF-15 level associated with a 72% higher (95% confidence interval, 1.21 to 4.45; P=0.003) and 65% higher (95% confidence interval, 1.08 to 2.50; P=0.02) risk of progression of kidney disease in C-PROBE and SKS participants, respectively. These results show that circulating GDF-15 levels strongly correlated with intrarenal expression of GDF15 and significantly associated with increased risk of CKD progression in two independent cohorts. Circulating GDF-15 may be a marker for intrarenal GDF15-related signaling pathways associated with CKD and CKD progression.


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Progression of kidney disease to ESRD is a major complication of CKD. The pathogenesis of progression of CKD is complex, and likely characterized by a constellation of pathologic features, including tubular atrophy and fibrosis, that progress independent of the primary cause of CKD. The discovery of biomarkers that identify new biologic pathways contributing to kidney disease progression is important.

Growth differentiation factor–15 (GDF-15) is a member of the TGF-β cytokine superfamily whose expression is increased in response to tissue ischemia, neurohormones, and other proinflammatory cytokines.1–3 Several studies have found a link between elevated levels of GDF-15 and cardiovascular injury in humans.4–8 It is also plausible that GDF-15 could signal intrarenal injury, either from a systemic process (e.g., cardiovascular disease) or an intrarenal pathophysiology. Few studies have examined the association of GDF-15 with progression of kidney disease. One study reported a significant association between elevations in GDF-15 and development of incident CKD, rapid decline of kidney function, and albuminuria among participants in the Framingham study.9 This study was limited to participants without CKD at study entry and thus did not study the CKD population, who are at the greatest risk of adverse kidney consequences.

Between two well-characterized longitudinal cohorts of CKD patients (the Clinical Phenotyping and Resource Biobank [C-PROBE] study and the Seattle Kidney Study [SKS]), we examined the association of circulating GDF-15 with intrarenal gene expression of GDF15 and with longitudinal progression of CKD. We hypothesized that elevation in serum GDF-15 would correlate with intrarenal tissue GDF15 expression and also be independently associated with loss of kidney function among CKD patients.

RESULTS

Characteristics of Study Populations
Mean ages of the participants from C-PROBE and SKS were 58 and 62 years, respectively. There were more women and black participants in C-PROBE compared with SKS: 60% versus 17%, and 41% versus 25%, whereas diabetes was more common in SKS (56% versus 39% in C-PROBE). Mean eGFR was 39.4 and 41.8 ml/min per 1.73 m², and median urine ACR was 135 and 287 mg/g, for SKS and C-PROBE respectively. Across quartiles of baseline GDF-15, there was a graded increase in annual decline in eGFR in univariate and multivariable models. Participants in the highest quartile of GDF-15 had a decline of 11.82% (95% CI, 0.8 to 21.61) per year in C-PROBE and a decline of 5.83% (95% CI, 0.13 to 11.53) per year in SKS. Every doubling of GDF-15 was associated with a 5.84% (95% CI, 1.84 to 9.68) or two-fold increased risk in SKS (hazard ratio, 2.56; 95% CI, 1.15 to 5.72) of 30% decline in eGFR or ESRD. Participants in the highest quartile of GDF-15 (versus the lowest quartile) had greater than three-fold in C-PROBE (hazard ratio, 3.25; 95% confidence interval [95% CI], 1.26 to 8.36) or two-fold increased risk in SKS (hazard ratio, 2.56; 95% CI, 1.15 to 5.72) of 30% decline in eGFR or progression to ESRD. Every doubling of GDF-15 was significantly associated with 72% (95% CI, 21% to 445%) or 65% (95% CI, 8% to 250%) greater risk of 30% decline in eGFR or progression to ESRD in C-PROBE and SKS, respectively (Table 2).

Correlation of Plasma GDF-15 with Intrarenal Expression of GDF15 in C-PROBE
In the C-PROBE cohort, 24 patients had matching plasma samples and gene expression data derived from kidney biopsy samples. We observed a significant inverse correlation between tubulointerstitial GDF15 mRNA expression with eGFR ($r = -0.47, P = 0.02$) (Figure 1A). The tubulointerstitial GDF15 mRNA was strongly and positively correlated with circulating GDF-15 protein ($r = 0.54, P = 0.01$) (Figure 1B).

Association of Circulating GDF-15 with 30% Decline in eGFR or Progression to ESRD in C-PROBE and SKS
In C-PROBE, the median follow-up time was 1.8 years with a median of two follow-up eGFR measures. In SKS, the median follow-up time was 2.7 years with a median of four follow-up eGFR measures. In C-PROBE, 72 (32.1%) participants progressed to the composite end point (23 developed ESRD and 49 had a 30% decline in eGFR). In SKS, 94 (32.0%) participants progressed to composite end point (27 developed ESRD and 67 had a 30% decline in eGFR). Incidence rates for adverse kidney outcomes were higher among participants with greater concentrations of GDF-15 (Table 2). After adjustment for demographics, kidney function, and comorbidity, GDF-15 was significantly associated with greater risk of 30% decline in eGFR or ESRD. Participants in the highest quartile of GDF-15 (versus the lowest quartile) had greater than three-fold in C-PROBE (hazard ratio, 3.25; 95% confidence interval [95% CI], 1.26 to 8.36) or two-fold increased risk in SKS (hazard ratio, 2.56; 95% CI, 1.15 to 5.72) of 30% decline in eGFR or progression to ESRD. Every doubling of GDF-15 was significantly associated with 72% (95% CI, 21% to 445%) or 65% (95% CI, 8% to 250%) greater risk of 30% decline in eGFR or progression to ESRD in C-PROBE and SKS, respectively (Table 2).

Association of Circulating GDF-15 with Annualized Relative Change in eGFR in C-PROBE and SKS
In both C-PROBE and SKS, participants with higher GDF-15 had greater risk of longitudinal decline in eGFR (Table 3). Across quartiles of baseline GDF-15, there was a graded increase in annual decline in eGFR in univariate and multivariable models. Participants in the highest quartile of GDF-15 had a decline of 11.82% (95% CI, 0.8 to 21.61) per year in C-PROBE and a decline of 5.83% (95% CI, 0.13 to 11.53) per year in SKS. Every doubling of GDF-15 was associated with a 5.84% (95% CI, 1.84 to 9.68) and 3.01% (95% CI, 0.74 to 5.29) decline in eGFR per year in C-PROBE and SKS, respectively (Table 3).

DISCUSSION
In this study of two independent, well-characterized CKD cohorts, we found intrarenal GDF15 expression in the tubulointerstitial compartment of patients with CKD is reflected by circulating GDF-15 levels, which are significantly associated with disease progression, as defined by continuous decline of eGFR or reaching a composite end point of 30% decline in eGFR or progression to ESRD, independent of other risk factors for progression of kidney disease, including...
Table 1. Baseline characteristics of study population by baseline serum concentrations of GDF-15 (pg/ml)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C-PROBE Study (n=224)</th>
<th>SKS (n=297)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>GDF-15 Q1 1306.8–436.8 pg/ml</td>
</tr>
<tr>
<td>Number of participants</td>
<td>224</td>
<td>293</td>
</tr>
<tr>
<td>Age, yr</td>
<td>58 (15)</td>
<td>53 (14)</td>
</tr>
<tr>
<td>Women, %</td>
<td>135 (60)</td>
<td>37 (66.1)</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>122 (55.2)</td>
<td>22 (39.3)</td>
</tr>
<tr>
<td>Black</td>
<td>90 (40.7)</td>
<td>31 (55.4)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (4.1)</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>Education, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Some high school or less</td>
<td>24 (10.9)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>Completed high school</td>
<td>132 (60.0)</td>
<td>31 (56.4)</td>
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<tr>
<td>Completed college</td>
<td>64 (29.1)</td>
<td>22 (40.0)</td>
</tr>
<tr>
<td>Prevalent disease, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>35 (15.6)</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>18 (8.0)</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>87 (38.8)</td>
<td>17 (30.4)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>193 (86.2)</td>
<td>44 (78.6)</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>28 (12.5)</td>
<td>7 (12.5)</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>136 (21)</td>
<td>128 (15)</td>
</tr>
<tr>
<td>eGFR, ml/min per 1.73 m²</td>
<td>41.8 (18.5)</td>
<td>55.7 (18.9)</td>
</tr>
<tr>
<td>Urine albumin-to-creatinine ratio, median (IQR)</td>
<td>286.5 (24.7, 1312.4)</td>
<td>104.8 (13.2, 593.9)</td>
</tr>
<tr>
<td>Antihypertensive medication use, %</td>
<td>145 (64.7)</td>
<td>37 (66.1)</td>
</tr>
</tbody>
</table>

Data are displayed as mean (SD) unless noted otherwise. Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; IQR, interquartile range.

*Race: n=221, not available=3.

Educational attainment: n=220, not available=4.
GDF-15. These tissue injury.3 In the heart, GDF-15 expression activates pathways responsible for cardioprotection.1–3 In a study of rat ventricular cardiomyocytes, GDF-15 expression increased 3 hours after ischemia.13 In GDF-15 knock-out mice, cardiac infarct area was larger compared with wild-type mice, supporting the hypothesis that GDF-15 activates cardioprotective pathways.13

Preclinical studies show significant protective effects of GDF-15 in the kidney as well. In mouse models of type 1 and type 2 diabetes, GDF-15 knock-out mice displayed increased tubular damage, with evidence of glucosuria and polyuria, as well as increased interstitial damage, as indicated by increased α-smooth muscle actin staining and collagen type 1 expression. Increased expression of GDF15 mRNA in outer medullary collecting duct cells has been reported during metabolic acidosis14 and potassium depletion,15 and both are associated with reduced kidney function. Data further suggest that GDF-15 not only can modulate the renal extracellular matrix production through an indirect route other than the Smad signaling pathway, but may also enhance cellular proliferation of tubular epithelial cells through noncanonical MAP kinase signaling pathway.16 In fact, a study demonstrated that the longitudinal proliferation of acid-secreting collecting duct cells was dependent upon expression of GDF-15,17 suggesting that GDF-15 may be crucial for maintaining tubular integrity by enhancing tubular repair. Intrarenal GDF15 may also prevent organ damage through controlling recruitment of inflammatory cells, as previously reported in experimental myocardial infarction and an early type 1 diabetic kidney disease model.13,16 Taking these experimental discoveries together with our findings, a plausible signaling pathway could be that kidney damage stimulated intrarenal GDF15 expression, possibly by TNF-α and p53-dependent and -independent mechanisms,18 which then triggered a series of protective responses including inhibiting extracellular matrix protein accumulation, activating proliferation of the tubular epithelial cells, and preventing inflammatory cell recruitment. Future studies are needed to determine if administration of GDF-15 is able to ameliorate pathologic changes in kidney disease using in vitro and in vivo models.

Our findings have important implications. First, GDF-15 may be an opportunity to expand the current panel of kidney biomarkers. There has been substantial interest in moving beyond eGFR and albuminuria to molecular biomarkers that may more specifically reflect pathophysiology involved in CKD progression. Second, elevations in GDF-15 may help identify CKD patients at highest risk for CKD progression. Third, these data may lead to further studies to understand the molecular mechanisms that underlie CKD progression and elevations in GDF-15, which may lead the way for novel therapeutics.

Our study had several strengths. We studied two independent, well-characterized CKD cohorts that represented a broad range of CKD in the United States across multiple geographic locations. A subset of the study population had kidney biopsies that allowed study of intrarenal GDF15 mRNA and plasma GDF-15. These findings suggest, for the first time, that intrarenal GDF15 mRNA, which is reflected by circulating levels of GDF-15, may be related to CKD progression.

GDF-15 is a member of the TGF-β cytokine superfamily that is widely expressed in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells and may reflect an early response protein induced after tissue injury.3 In the heart, GDF-15 expression activates pathways responsible for cardioprotection.1–3 In a study of rat ventricular cardiomyocytes, GDF-15 expression increased 3 hours after ischemia.13 In GDF-15 knock-out mice, cardiac infarct area was larger compared with wild-type mice, supporting the hypothesis that GDF-15 activates cardioprotective pathways.13

Preclinical studies show significant protective effects of GDF-15 in the kidney as well. In mouse models of type 1 and type 2 diabetes, GDF-15 knock-out mice displayed increased tubular damage, with evidence of glucosuria and polyuria,
were performed in different laboratories and years, however, the same assay was used for both cohorts. Included participants from both cohorts were older with lower baseline eGFR (compared with excluded participants), which may have influenced our findings. All eGFR measures to determine the outcomes of interest (>30% loss of baseline eGFR) were taken in the ambulatory setting and were on the basis of a single follow-up eGFR measure. It is possible that participants had fluctuations in eGFR or AKI. However, this would have likely biased our results toward the null. Ascertainment of covariates differed in the two independent CKD cohorts. Circulating GDF-15 was strongly correlated with intrarenal tubulointerstitial GDF15 expression. GDF-15 may inform intrarenal abnormalities that are associated with CKD and with CKD progression. Further studies should confirm and extend these findings.

**CONCISE METHODS**

**Study Populations**

**The C-PROBE Study**

The C-PROBE, supported by the George M. O’Brien Michigan Kidney Translational Core Center at the University of Michigan, is a multicenter prospective observational cohort study. Patients with CKD cohorts—C-PROBE relied on clinical databases whereas SKS relied on study visit information. We did not have longitudinal cystatin C measures in C-PROBE so eGFR was defined using serum creatinine. However, serum creatinine is the kidney biomarker most widely used clinically. Unfortunately, clinical or novel inflammatory markers were not systematically available in both C-PROBE and SKS and thus could not be assessed in this analysis. Although we found strong associations in our study, we cannot determine causality on the basis of our current data.

In conclusion, elevated GDF-15 was associated with greater than a two-fold increased risk of decline of eGFR in two independent CKD cohorts. Circulating GDF-15 was strongly correlated with intrarenal tubulointerstitial GDF15 expression. GDF-15 may inform intrarenal abnormalities that are associated with CKD and with CKD progression.
stage I–IV are recruited from six sites in the United States, including: John H. Stroger Hospital, Chicago; Renaissance Renal Research Institute, Detroit; University of Michigan Nephrology Program, Ann Arbor; Wayne State University Nephrology Program, Detroit; Temple University Nephrology Program, Philadelphia; and Carolinas Medical Center, Levine Children’s Hospital, Charlotte (http://kidneycenter.med. umich.edu/cemplace).15,16 Patient demographics, socioeconomic variables, clinical information, and biosamples are collected at enrollment and annually thereafter. Patients with polycystic kidney disease and those who underwent dialysis or transplantation were excluded. At time of this study, 741 CKD participants were enrolled in C-PROBE.

Inclusion criteria for this study were age >18 years and baseline eGFR ≤ 90 ml/min per 1.73 m², and patients have at least three eGFR values during the observation period (including retrospective eGFR values before study entry and/or prospective eGFR measures). The inclusion of retrospective measures to select study participants was part of a larger study to examine novel biomarkers and kidney function decline.15 Participants also needed to have both plasma and urine samples available at study entry to be included in our study (n = 308). We excluded participants who did not have follow-up beyond the baseline study visit (n = 84), leaving a final analytic sample of n = 224. Participants who were included in the analysis were more likely to be older, and have lower eGFR and higher comorbidity (Supplemental Table 1). A subgroup of n = 24 of the 224 patients underwent kidney biopsies for clinical indications, which were used for gene expression analysis.

The SKS
The SKS is an ongoing nephrology clinic–based cohort study of people with CKD, which began recruitment in 2004 and study visits in 2005.17–20 Participants were recruited from outpatient nephrology clinics at the University of Washington Medical Center, Harborview Medical Center, and the Veterans Affairs Puget Sound Health Care Center in Seattle, Washington. Inclusion criteria were age >18 years, and eGFR ≤ 90 ml/min per 1.73 m² or a urinary protein-to-creatinine ratio of >30 mg/g. Exclusion criteria for entry into the cohort included: current dialysis, previous kidney transplantation, inability to provide informed consent, or expectation of dialysis initiation within 3 months. The study was approved by the University of Washington Institutional Review Board. The protocol includes annual in-person study visits with blood collections which are separate from clinical events and therefore reflective of ambulatory health status. A total of 530 participants met inclusion criteria and were enrolled in SKS.

For this study, we excluded participants who did not have follow-up beyond the baseline study visit (n = 111) or who did not have available serum for biomarker analysis (n = 126), leaving a final analytic sample of n = 297. Participants who were included in the analysis were more likely to be older, and have lower eGFR and higher comorbidity (Supplemental Table 1).

Circulating GDF-15 Measurement in C-PROBE and SKS
GDF-15 concentration was measured in plasma (C-PROBE) or serum (SKS) samples using Human GDF-15 Quantikine ELISA (R&D Systems, Minneapolis, MN) in both C-PROBE (2013) and SKS (2014). Assay validation was performed following standard operation protocol for conduct of immunoassay validation including dynamic concentration range, lower and upper limits of quantification, matrix effect, precision, stability, selectivity and specificity, and dilution parallelism. A dilution factor of 1:4 was applied for plasma samples. The absorbances were measured by spectrophotometry (450 nm) and unknown concentrations determined through a four-parameter logistic curve fit. In C-PROBE, all samples were measured in duplicate and means were used for further quantification. The intra- and interplate coefficients of variation of the QC samples were <10% in C-PROBE and in SKS.

Gene Expression Profiling in C-PROBE
To understand the underlying mechanism of circulating GDF-15 with progression of CKD, we investigated the association of circulating GDF-15 with intrarenal GDF15 expression as well as intrarenal GDF15 expression with eGFR. On the basis of RNAseq data derived from rat nephron segments,21 GDF15 RNA expression was detected in several tubular segments, including short descending limb, inner medulla long descending limb, cortical collecting duct, and inner medullary collecting duct (Supplemental Figure 1). Therefore, we specifically investigated tubulointerstitial expression of GDF15. Gene expression was performed on microdissected tubulointerstitial compartment from the kidney biopsy tissue of 24 (out of 224) C-PROBE patients. The details of tissue harvesting, microdissection, RNA isolation, reverse-transcription, linear amplification, and target preparation followed published strategies;15 Affymetrix GeneChip Human Genome–U133 Plus 2.0 Array were used for this study. The raw data were preprocessed, normalized, and log2-transformed.15

Kidney Outcomes
Serum creatinine was measured by the modified rate Jaffe method with an assay traceable to isotope dilution mass spectrometry. We calculated eGFR annually using the CKD-EPI equation.22 The primary renal outcome was the time to the first occurrence of ≥30% loss of baseline eGFR or initiation of renal replacement therapy (need for chronic dialysis or kidney transplantation) over follow-up. A secondary outcome was annualized relative change in eGFR over the follow-up period. End of follow-up was February 2013 for SKS and January 2014 for C-PROBE.

Covariates
Covariates were obtained from clinical records for participants in C-PROBE and from research study visits in SKS. Prevalent conditions were determined on the basis of physician diagnosis in the medical record (C-PROBE) or by self-report (SKS). In C-PROBE, medications were ascertained from the patients’ medical records. In SKS, medications were assessed by inventory assessment and missing medication data were completed by chart review.23 In SKS, diabetes by any of the following: use of an oral hypoglycemic medication or insulin, fasting blood sugar ≥ 126 mg/dl, nonfasting blood sugar ≥ 200 mg/dl, or hemoglobin A1c ≥ 6.5%. In SKS, three seated BP measurements were recorded using an automated sphygmomanometer; the average of the last two readings was retained for analysis. Hypertension was defined by the use of any antihypertensive medication, systolic BP ≥ 140 mmHg, or diastolic BP ≥ 90 mmHg. General chemistries were measured on a Beckman-Coulter DxC autoanalyzer (Beckman-Coulter, Brea, CA).
Urine albumin and creatinine were measured in spot morning or overnight urine collections, by a timed end point method and with the modified rate Jaffe method.

Statistical Analyses
Characteristics of the study populations in C-PROBE and SKS were described across study-specific quartiles of circulating GDF-15.

The correlations of intrarenal GDF15 transcript with circulating GDF-15 levels and baseline kidney function in matching samples were calculated using Pearson correlation.

We calculated unadjusted incidence rates as the number of events divided by person-years at risk. The functional form of the association of circulating GDF-15 with first occurrence of loss of >30% eGFR or initiation of RRT was assessed using cubic splines. The association was linear, so we conducted the analyses modeling GDF-15 in study-specific quartiles as well as a continuous exposure (per doubling). Cox proportional hazards were used to test the association of GDF-15 (in study-specific quartiles as well as continuous per doubling) measured at baseline and time until the first occurrence of loss of >30% eGFR or initiation of RRT. Covariates for adjustment were selected a priori on the basis of previous literature on potential confounders for the association of GDF-15 with study outcomes.9,11,12 Nested models were used in order to progressively explore the confounding effects of demographic characteristics, measures of kidney function, comorbidity, and medication use. The first model included baseline age, sex, race (white, black, other), eGFR (log-transformed), and urine albumin-to-creatinine ratio (log-transformed). The second model added prevalent cardiovascular disease, diabetes, systolic BP, and antihypertensive medication use. Participants were censored at death, loss to follow-up, or end of data collection (February of 2013 for SKS and January of 2014 for C-PROBE).

We also tested the association of baseline GDF-15 with annualized relative change in eGFR over the follow-up period using generalized estimating equation models, accounting for within-participant clustering across time. We adjusted for covariates as above. Among participants who initiated dialysis, we imputed an eGFR of 10 ml/min per 1.73 m². Patients were censored after progression to ESRD.

All P values were two-tailed (α=0.05). All analyses were performed using Stata release 13.1 (College Station, TX) and R.

ACKNOWLEDGMENTS

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DISCLOSURES

M.C.M. and I.F. are employees of Astrazeneca and hold AstraZeneca shares. M.B and L.E are permanent employees of Hoffmann-La Roche. Unrelated to this manuscript, D.G. serves as a consultant through industry to University of Michigan agreements with Janssen, Bristol-Myers Squibb, and Dimerix industries and has research funding from NephCure Kidney International (nonprofit patient advocacy) and Retrophin (industry). M.K. and F.B. have no consultancy with Lilly and Co. through the University of Michigan with no personal financial remuneration.

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