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# **BMJ Open**

# Increased serum REG Ia is associated with eGFR decline in patients with chronic kidney disease

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36	Abstract
37	Objective
38	This study conducted to demonstrate the relationship between levels of serum REG I $\alpha$
39	and eGFR and to explore the efficiency of REG I $\alpha$ in CKD detection.
40	Design
41	A cross-sectional study.
42	Setting
43	Conducted in Zhongda Hospital between August 2022 and August 2023.
44	Participants
45	880 participants were enrolled in this study, with 220 non-CKD participants and 660
46	patients with CKD.
47	Methods
48	Correlation analyses were conducted to determine the association between REG I $\alpha$ and
49	kidney function. Receiver operating characteristic curves (ROC) were plotted to assess
50	the ability of serum REG I $\alpha$ in screening patients with CKD.
51	Results
52	In CKD group, the levels of serum REG Ia (125.54 [60.28-303.39] ng/mL) were
53	significantly higher compared to those in non-CKD group (24.62 [14.09-37.32] ng/mL,
54	$P < 0.001$ ). Serum REG I $\alpha$ exhibited a positive relationship with serum creatinine (Scr),
55	cystatin C (Cys-C), and kidney injury molecular-1(KIM-1), while showed a negative
56	relationship with eGFR. The regression analysis revealed a significant association
57	between serum REG I $\alpha$ and eGFR (OR=1.737 [1.263-2.388], $P = 0.001$ ). Furthermore,
58	levels of serum REG I $\alpha$ were found to gradually increase with the decline of kidney
59	function ( $P < 0.001$ ). Serum REG I $\alpha$ was recognized to play a role in screening CKD
60	patients, with an AUC of 0.860 (0.813-0.899), providing a sensitivity of 71.63%, a
61	specificity of 86.89%, a PPV of 94.30%, and an NPV of 46.85%. Additionally, serum
62	REG I $\alpha$ had an AUC of 0.769 (0.712-0.819) in screening patients at high and very-high
63	risk for CKD according to KDIGO risk stratification, exhibiting significantly higher
64	sensitivity compared to serum Cys-C and KIM-1 (82.80% vs 75.16% and 36.94%).

65	Conclusions
66	This study provided evidence that levels of serum REG I $\alpha$ were notably elevated in
67	patients with CKD and exhibited a strong association with kidney function. The REG
68	Iα might serve as an important biomarker for CKD.
69	
70	Trial registration
71	The study was approved by the ethics committee of Zhongda Hospital
72	(2022ZDSYLL204-P01) and was in compliance with the Helsinki Declaration. The
73	study had a clinical study registration number of ChiCTR2300072247.
74	Data availability statement
75	The data underlying this article is available from the corresponding author under
76	reasonable request.
77	Patient and public involvement statement
78	It was not appropriate or possible to involve patients or the public in the design, or
79	conduct, or reporting, or dissemination plans of our research.
80	
81	Strengths and limitations of this study
82	• We utilized different logistic regression analyses to adjust for various confounding
83	factors and investigate the relationship between serum REG I $\alpha$ and kidney function.
84	• This study was the first time to apply KDIGO risk stratification for subgrouping in
85	the CKD population, analyzing the potential predictive ability of serum REG I $\alpha$ in
86	renal function decline and the risk of CKD progression.
87	• Although potential causal relationships can be identified through regression model
88	analyses in this cross-sectional assessment, further prospective cohort follow-up is
89	necessary to offer a more comprehensive understanding.
90	• Our survey did not definitively identify the exact source of elevated REG I $\alpha$ in
91	patients with CKD. Therefore, further mechanistic studies should be conducted to
92	investigate the origins of REG I $\alpha$ in the situation of kidney impairment.
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- 100 **Competing interests statement** 
  - 101 The authors declare that they have no competing interests.
- **Keywords** 103

κidney . Regenerating protein Ia, Chronic kidney disease, Biomarker, Kidney function, Risk 104

105 stratification;

List of abbreviations	
Regenerating protein Ia	REG Ia
Diabetic kidney disease	DKD
Chronic kidney disease	CKD
Receiver operating characteristic curves	ROC
Cystatin C	Cys-C
Kidney injury molecular-1	KIM-1
Area under the ROC	AUC
End stage kidney disease	ESKD
Serum creatinine	Scr
Blood urea nitrogen	BUN
Estimate glomerular filtration rate	eGFR
Urine albuminuria creatine ratio	UACR
Kidney disease improving global outcomes	KDIGO
Pancreatic stone protein	PSP
Chronic kidney disease epidemiology collaboration	CKD-EP
Unilateral ureteral obstruction	UUO
Uric acid	UA
Fast blood glucose	FBG
Cardiovascular disease	CVD
Standard deviation	SD

# 109 Introduction

110 Chronic kidney disease (CKD) has a broad range of underlying etiologies and variable 111 progression rates, <sup>1,2</sup> and may become the fifth leading cause of death worldwide by 112 2040.<sup>3</sup> The endpoint of CKD is end stage kidney disease (ESKD) in which 90% of 113 kidney function reduced, making it impossible to sustain life over the long term.<sup>2</sup> The 114 high prevalence, low detection rate, severe outcomes, and substantial medical costs of 115 CKD make it a significant global health concern.<sup>4</sup> Early prevention, detection, and 116 treatment can lead to better outcomes and prevent ESKD progression.

Biomarkers such as serum creatinine (Scr), blood urea nitrogen (BUN), estimate glomerular filtration rate (eGFR), and urine albuminuria creatine ratio (UACR) are routinely used to assess the severity of CKD.<sup>5-8</sup> In 2012, Kidney Disease: Improving Global Outcomes (KDIGO) has taken the eGFR and UACR to create risk stratification strategies, assisting in the tailored management of CKD and guiding treatment for those at a higher risk of progression.<sup>9</sup> New biomarkers like, cystatin C (Cys-C), kidney injury molecular-1 (KIM-1), and β2-microglobuline have shown great application potential in enhancing the precision of early-stage CKD screening, either independently or in conjunction.<sup>2,3,10,11</sup> However, most biomarkers failed to match clinical expectations.<sup>12-</sup> <sup>15</sup> Few biomarkers can both detect and assess risk stratification effectively in patients with CKD. Thus, discovering a biomarker can screen kidney function decline and evaluate CKD progression risk is crucial.

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Regenerating protein I $\alpha$  (REG I $\alpha$ ) is a 16 kDa protein primarily secreted by the pancreas and intestine.<sup>16</sup> also known as pancreatic stone protein (PSP).<sup>17</sup> The involvement of REG I $\alpha$  plays a role in the processes of cellular proliferation and regeneration.<sup>18,19</sup> Recent researchers have identified the presence of REG Ia in patients with various kidney diseases.<sup>20,21</sup> Our previous studies also have indicated that serum levels of REG Iα elevate in patients with diabetic kidney disease (DKD), which are concordant with the finding of H. Sobajima.<sup>22-24</sup> These evidences indicate that REG Iα may serve as a biomarker of kidney insufficiency. 

137 In this study, we analyzed the association between serum REG I $\alpha$  levels and kidney

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function, and further assessed its potential as a screening tool for CKD and as abiomarker of kidney function.

# 141 Study subjects

The participants were enrolled from Zhongda Hospital between August 2022 and
August 2023. The study was approved by the ethics committee (2022ZDSYLL204P01), with a clinical study registration of ChiCTR2300072247. Informed consent was
acquired from all participants.

The inclusion criteria were as follows: (1) non-CKD participant: age older than 18 years; (2) CKD patients: age older than 18 years and diagnosed with CKD in accordance with the guidelines in 2012.<sup>1</sup> The exclusion criteria were as follows: (1) enrolled in another trial; (2) acute kidney injury; (3) patients with ESKD who are undergoing renal replacement therapy; (4) pregnancy; (5) active infection; (6) acute or chronic inflammation of the gastrointestinal system and pancreas; (7) history of gastrointestinal or pancreatic resections; (8) cancer; (9) mental disorders.

eGFR was calculated in accordance with the Chronic Kidney Disease Epidemiology
Collaboration (CKD-EPI) equation.<sup>25</sup> CKD groups was classified using the method
proposed by the U.S. National Kidney Foundation. The risk stratification of CKD
progression was defined in accordance with KDIGO risk stratification guideline.<sup>26,27</sup>

157 Data collection and quality assessment

Demographics information was collected at baseline through questionnaires. All participants have undergone 12-h fasting and taken about 3mL of peripheral blood sample. The blood samples were centrifuged directly for 15 min at a rotating speed of 3,500 rpm, and the upper serum was sucked up within 6-h. The serum was immediately frozen in refrigerator (-80°C). Clinical biochemical parameters were extracted from the clinical laboratory of Hospital, such as Scr, BUN, uric acid (UA), Cys-C, Myoglobin, UACR, and fast blood glucose (FBG). The laboratory implements internal and external quality control procedures directed by a Chinese Quality Control Laboratory. The serum REG Ia levels were determined by a double antibody sandwich ELISA, as

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previously described.<sup>28</sup> Serum KIM-1 was detected by an ELISA kit (KE00136) from Proteintech.

#### **Statistical analysis**

Statistical analyses were conducted using SPSS 20.0, Med-Calc, and GraphPad Prism 8.0. Continuous data with normal distribution were summarized as mean  $\pm$  standard deviation (SD), otherwise as median with interquartile range. For categorical variables, the frequency with a percentage of each category was calculated. Spearman's rank correlation analyses and ordinal logistic regression was used to measure the associations between serum REG Ia and other biomarkers of kidney function. Multivariate logistic regression analysis was utilized to identify the independent factors of kidney dysfunction. The area under the receiver operating characteristic curve (AUC) was plotted to analyze the ability of serum Cys-C, KIM-1, and REG Iα to screen the patients with CKD, and detect the high and very-high risk patients. A P value of <0.050 using two-tailed tests was considered statistically significant. 

Results 

#### **Baseline characteristics of the study population**

Overall, 880 participants were finally enrolled, with 220 non-CKD participants and 660 patients with CKD (Supplementary data Figure 1). Differences in age, complication diseases (diabetes and hypertension), kidney function biomarkers (Scr, BUN, UA, eGFR, Cys-C, and KIM-1), and serum myoglobin were observed between patients with CKD and non-CKD participants (all P < 0.001, Table 1). Serum REG Ia levels were notably higher in patients with CKD (125.54 [60.28-303.39] ng/mL), than in non-CKD participants (24.62[14.09-37.32] ng/mL, P < 0.001, Table 1). Serum Cys-C, REG Ia, and myoglobin showed increasing trends as eGFR levels decreased (Figure 1: A1, C1, and D1). There were significant differences in serum REG Ia among each group (P <0.001, Figure 1: C1). A significant difference in serum KIM-1 was found between the non-CKD group and G1 group (P < 0.010, Figure 1: B1). Two reanalyzes balanced the effects of CVD on the distribution of serum REG Ia and myoglobin (supplementary data Figure 2), and the effects of diabetes on patients with CKD (Supplementary data Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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196 Figure 3).

# 197 Relationship between serum REG Iα and kidney function

This study explored the relationship between serum REG Ia levels and kidney function biomarkers. In figure 2, Spearman correlation analyses demonstrated that serum REG Ia was positively correlated with Scr (r = 0.753, P < 0.001), BUN (r = 0.733, P < 0.001), serum Cys-C (r = 0.678, P < 0.001), and serum KIM-1 (r = 0.217, P < 0.001), and negatively correlated with eGFR (r = -0.789, P < 0.001). The comprehensive correlations between serum REG Ia and various markers were presented in supplementary data Table 1. In ordinal logistic regression analysis, eGFR values were used as a grade-dependent variable in the model. The results showed that serum REG  $I\alpha/100$  levels was markedly associated with eGFR (OR=1.737 95% CI: 1.263-2.388, P = 0.001, Table 2).

# 208 Subgroup analysis in patients with CKD

To investigate the relationship between serum REG Ia and the risk of CKD progression, 256 CKD patients were included in the sub-research and were classified by eGFR and UACR levels in accordance with KDIGO risk stratification guideline.<sup>1</sup> The patients were divided into four groups to assess the progression and prognostic, namely, low risk (18.00%), moderate risk (20.70%), high risk (17.20%) and very-high risk (44.10%, supplementary data Table 2). We found that levels of serum Cys-C elevate gradually from 1.22 (1.01, 1.43) mg/L in the moderate risk group to 2.57 (1.94, 3.53) mg/L in the very-high risk group (P < 0.010), however, no significant difference was found between the low risk group and moderate risk group (P > 0.050, Figure 1: A2). The levels of serum REG Ia elevate gradually from 48.86 (34.18-78.28) ng/mL in the low risk group to 184.38 (108.81-314.71) ng/mL in the very-high risk group (P < 0.010). Significant differences were observed in serum REG Ia levels among the groups (P < 0.010), except for the moderate risk group and high risk group (P > 0.050, Figure 1: C2). The very-high risk group had the highest serum myoglobin level among the four groups (P <0.010), however, there were no significant differences among other three groups (P >0.050, Figure 1: D2).

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 The multiple logistic regression analysis demonstrated that serum REG I $\alpha$ /100 was an independent influencing factor for patients with CKD who are at high and very-high risk (OR=1.799, 95% CI: 1.088-2.975, *P* = 0.022, Table 2).

# 228 Ability of serum REG Iα in screening patients with kidney dysfunction

In evaluating the potential application of serum REG Ia as a screening tool for patients with CKD and its ability to differentiate the risk stratification, we performed receiver operating characteristic curve (ROC) analyses (Figure 3 and supplementary data Table 3). The AUC for serum REG Ia to detect patients with CKD was 0.860 (95% CI: 0.813-0.899) as well as serum creatinine (0.850, 95% CI: 0.801-0.890) and Cys-C (0.842, 95% CI: 0.793-0.883), with a cutoff value of 70.82 ng/mL, sensitivity of 71.63%, specificity of 86.89%, positive predictive value (PPV) of 94.30%, and negative predictive value (NPV) of 46.85%. By contrast, serum KIM-1 had a lower AUC than serum REG Ia, measuring 0.714 (95% CI: 0.656-0.767, P < 0.001). Serum myoglobin had the lowest AUC among the five biomarkers, measuring 0.642 (95% CI: 0.581-0.699, P < 0.001). Furthermore, we analyzed the ability of serum Cys-C, KIM-1, and REG I $\alpha$  in distinguishing high and very-high risk patients with CKD in accordance with the KDIGO risk stratification. As shown in supplementary data Table 3, serum Cys-C had an AUC of 0.865 (95% CI: 0.817-0.904, P < 0.010), which was the highest AUC among the three markers. Serum REG Ia had an AUC of 0.769 (95% CI: 0.712-0.819), showing better performance than serum KIM-1 (P < 0.010). The cutoff value of serum REG Ia was 76.05 ng/mL, and the sensitivity, specificity, PPV, and NPV were 82.80%, 62.63%, 77.38%, and 69.32%, respectively. Notably, serum REG Iα had a significantly higher sensitivity than serum Cys-C and KIM-1. 

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248 Discussion

This study was the first time to investigate the whole CKD population, and confirm the
upregulation of serum REG Iα in patients with CKD. Initially, the serum levels of REG
Iα showed a gradually increasing trend with the decrease of eGFR. Second, serum REG
Iα was negatively correlated with eGFR and positively correlated with Scr, BUN, serum
Cys-C, and serum KIM-1 levels. Third, serum REG Iα was an independent risk factor

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for high and very-high risk patients in accordance with the KDIGO risk stratification.
Finally, serum REG Iα played an important role in screening patients with CKD and
detecting high and very-high risk patients in CKD.

REG I $\alpha$  was a low molecular weight (16 kDa) protein initially discovered in pancreas, where it exhibited a remarkable ability to inhibit the formation of calcium carbonate stones in pancreatic ducts.<sup>29</sup> Hence, REG Ia was also called pancreatic stone protein (PSP) at the time.<sup>30</sup> Immunocytochemical analyses indicated that REG Ia protein was overexpressed in impaired kidney, with predominant localization observed in proximal tubules and thick ascending limbs of Henle's loops.<sup>20</sup> Then, some studies declared that urine PSP (REG Ia) and REG Ia gene might indicate kidney tubular dysfunction and potentially serve as an early biomarker of DKD detection and progression.<sup>22,31,32</sup> Our previous studies provided compelling evidence, that serum REG Iα levels were notably elevated in patients with type 2 diabetes mellitus who had kidney dysfunction. And we also observed that pregnant women with early renal dysfunction exhibited similar elevations in serum REG Ia levels.<sup>24,33</sup> Notably, the serum REG Ia levels remained significantly elevated when the scope of this study was expanded to the entire CKD population. 

We incorporated myoglobin (17.6 kDa)<sup>34</sup> to investigate whether the elevated level of serum REG Ia represents a universal phenomenon of accumulation in CKD. The myoglobin was identified as a biomarker for acute myocardial ischemia and rhabdomyolysis.<sup>35</sup> Some studies indicated that serum myoglobin levels increase in kidney impairment. This accumulation was primarily attributed to slower clearance in CKD.<sup>36</sup> We showed that serum levels of myoglobin have no differences between non-CKD participants and patients with early stage of CKD (group G1 and group G2). And there was a gradual increase in patients with medium to end-stages of CKD (group G3 to group G5). The possible reason was as follows: in the early stages of kidney injury, compensatory glomerular hypertrophy with hyperfiltration state was known to exist. This mechanism allowed some low molecular weight protein to be filtered through the glomerular basement membrane, resulting in no discernible difference during the early

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stage of CKD (eGFR > 60 mL/min per 1.73 m<sup>2</sup>), and even appearing as a transient decrease. With progression to medium to end-stages of CKD (eGFR < 60 mL/min per 1.73 m<sup>2</sup>), the glomerular basement membrane thickened and led to significant decline in glomerular filtration function, ultimately causing proteins accumulation in serum.<sup>37</sup> The serum levels of REG Ia showed a gradual increase in medium to end-stages of CKD, which was similar to the rise observed in serum myoglobin levels. However, it exhibited a significant increase in the early stage of CKD, which was different with serum myoglobin. This suggested that, in addition to the sensitive accumulation of serum REG I $\alpha$  as a low molecular weight protein, there might be a specific production when it comes to kidney impairment. 

The etiology underlying the upregulation of REG Ia production in patients with CKD remains elusive. Two potential mechanisms are considered as follows. First, REG Ia resists apoptosis and promotes cell proliferation in different inflammation situations.<sup>38-</sup> <sup>42</sup> Studies have shown that cytokines such as IL-6 can increase the proliferation of REG Ia, which is involved in cell regeneration and repair.<sup>42,43</sup> In CKD, the occurrence and progression of the disease are closely related to the expression of multiple inflammatory cytokines, including IL-1, IL-6, and TNF- $\alpha$ . <sup>37,44,45</sup> Under the stimulation of chronic inflammation, different types of renal cells secrete REG Ia locally to participate in kidney anti-apoptosis and proliferation and against kidney fibrosis in the development of CKD. The secreted REG Ia enters the circulation through reabsorption by kidney tubules, resulting in a significant increase in serum REG Ia levels. Thus, REG Ia might serve as an inflammatory factor involved in kidney diseases. Second, REG Ia is primarily synthesized in the pancreas and released into the circulation.<sup>18,46</sup> A hypothesis suggests that a cross-talk mechanism exists between the pancreas and kidney when renal damage occurs. This interaction may prompt the pancreas to produce more REG I $\alpha$  in response to kidney injury, resulting the elevation in serum. Although the exact cause of REG Ia upregulation remains unclear, these two proposed mechanisms provided potential insights into the relationship between REG Ia and CKD. 

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At present, the assessment of CKD generally focused on glomerular filtration capacity,

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which is characterized by Scr, UACR, and eGFR.<sup>2</sup> The stabilities of these factors are compromised by age, dietary intake, and physical activity. In general, the performance of eGFR is poor for populations outside of North America, Australia, and Europe, and accuracy decreases at the extremes in the distribution of age, and body composition.<sup>7</sup> Therefore, many new biomarkers of kidney impairment have been discovered. Cys-C is produced by all nucleated cells at a steady rate and freely filtered by the kidney.<sup>47</sup> The accumulation of its levels is observed in case of glomerular filtration dysfunction, with limited impact and strong stability.<sup>48</sup> Eventually, serum Cys-C is incorporated into the eGFR equation as a reliable and sensitive indicator of kidney function. The sensitivity of serum Cys-C is heightened in the early stages of CKD compared to creatinine.<sup>49,50</sup> Another biomarker is KIM-1,<sup>51,52</sup> which is secreted following kidney proximal tubular injury, and has emerged as a sensitive and specific biomarker of acute kidney injury and CKD progression.<sup>53-56</sup> In this study, serum REG Ia strongly correlated with serum creatinine and Cys-C, and had a similar performance to serum creatinine and Cys-C in detecting patients with CKD. The serum levels of REG Ia were more likely related to glomerular filtration capacity according to the correlation analyses, although the mechanism of its production and degradation or excretion in CKD remained unclear. We indicated that serum REG Ia might be more sensitive than serum creatinine and Cys-C in detecting ultra-early stage of kidney dysfunction. Similar phenomenon was observed in distribution analyses in KDIGO risk stratification. The KDIGO risk classification was widely used in evaluating the progression and prognosis risk in patients with CKD.<sup>57-60</sup> Serum levels of REG Ia gradually elevated with higher KDIGO risk stratification categories. And it emerged as an independent risk factor for patients with CKD categorized as high and very-high risk. Our findings provided a new insight that serum REG Ia performed better than serum KIM-1 in screening patients with CKD and detecting high and very-high risk patients with CKD. Moreover, serum REG Iα displayed a highest sensitivity in identifying high and very-high risk patients with CKD. These results highlight the potential application of serum REG Ia as a valuable biomarker in the screening of patients with CKD and the assessment of CKD

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risk. We observed that patients with CKD were older than non-CKD participants, and they had higher rates of diabetes and hypertension. This finding was consistent with the typical etiology of CKD. Globally, diabetes and hypertension are recognized as the primary causes of CKD.<sup>2</sup> Diabetes accounts for 30-50% of all CKD cases and affects approximately 285 million adults worldwide. A consistent finding in observational studies shows that the increased risk of developing is associated with blood pressure control. These findings were consistent with the expected proportion of CKD etiology in our study. 

There are some limitations in this study. First, it is a cross-sectional assessment and further follow-up studies must be conducted to provide a more comprehensive understanding. Second, although robust biomarkers such as creatinine and Cys-C are already available for the diagnosis of CKD, the potential efficacy of REG Ia as a combined biomarker is anticipated to be revealed in subsequent large-scale analyses. Third, this study did not definitively identify the exact source of elevated REG Ia in patients with CKD. Therefore, further mechanistic studies should be conducted to investigate the origins of REG Ia in the situation of kidney impairment. 

## 358 Conclusion

359 This study provided compelling evidence that serum REG Iα was significantly
360 upregulated in the patients with CKD and strongly associated with kidney function.
361 Serum REG Iα emerged as a significant biomarker for detecting kidney function decline.

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# 363 Authors' contributions

Ling Li conceptualized the study; Rolf Graf provided the excellent technical support for this study; Bin Wang reviewed and edited the manuscript; Nan Huang and Xiangyun Zhu were responsible for the data analysis and wrote the original draft. Nan Huang, Sheng Chen, Xiaodong Wu, Hui Wang, Zhiyi Shu and Xi Huang were responsible for inclusion of population, collection of samples and data, and the experiments. Jianling Bai and Jinfang Sun were responsible for guidance on data analysis. Pingsheng Chen and Xiuxiu Hu were responsible for analysis of kidney biopsy tissues from CKD patients.

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	Non-CKD Participants	<b>CKD</b> Patients	P Valu
Number	220	660	-
DEMOGRAPHICS			
Age (years)	53 (40-62)	62 (50-72)	< 0.001
Sex (male, %)	111 (50.45)	366 (55.45)	0.212
Smoking (yes, %)	41 (18.64)	142 (21.52)	0.141
Drinking (yes, %)	30 (13.64)	84 (12.73)	0.823
COMPLICATION DISEASES			
Diabetes (yes, %)	75 (34.09)	350 (53.03)	< 0.00
Hypertension (yes, %)	65 (29.55)	501 (75.91)	< 0.00
Cardiovascular disease (CVD, yes, %)	44 (20.00)	149 (22.58)	0.453
LABORATORY MEASUREMENTS			
Fast blood glucose (FBG, mmol/L)	5.45 (4.99-6.67)	5.34 (4.57-7.11)	0.008
Serum creatinine (Scr, µmol/L)	64.00 (55.25-76.00)	126.00 (83.00-418.50)	< 0.00
Blood urea nitrogen (BUN, mmol/L)	5.20 (4.30-6.28)	10.30 (7.00-18.60)	< 0.00
Serum uric acid (UA, µmol/L)	301.00 (256.00-365.25)	354.00 (290.00-443.00)	< 0.00
Estimated glomerular filtration rate (eGFR, mL/min per 1.73 m <sup>2</sup> )	102.37 (95.73-112.36)	44.03 (10.96-77.09)	< 0.00
Serum cystatin C (Cys-C, mg/L)	1.10 (0.99-1.24)	1.79 (1.23-3.50)	< 0.00
Serum kidney injury molecular-1 (KIM-1, pg/mL)	186.28 (57.22-266.88)	247.72 (175.10-334.13)	< 0.00
Serum Myoglobin (ng/mL)	46.81 (33.49-58.00)	64.04 (35.96-112.51)	< 0.00
Serum regenerating protein Iα (REG Iα, ng/mL)	24.62 (14.09-37.32)	125.54 (60.28-303.39)	< 0.00

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Table 2. The logistic regression analyses showing the relationship between variables

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	Ordinal	Logistic Regression <sup>#</sup>	Multivaria	te Logistic Regression <sup>3</sup>
	P Value	OR (95% CI)	P Value	OR (95% CI)
Age <sup>a</sup>	0.050	1.020 (1.001-1.041)	0.009	0.966 (0.942-0.992)
BUN <sup>b</sup>	< 0.001	1.266 (1.165-1.376)	< 0.001	1.440 (1.216-1.706)
UA¢	0.337	1.001 (0.999-1.004)	0.085	1.003 (1.000-1.007)
Serum Myoglobin <sup>d</sup>	0.165	1.005 (0.998-1.013)	N/A	N/A
Serum Cys-C <sup>e</sup>	< 0.001	6.784 (4.016-11.460)	0.071	1.853 (0.949-3.620)
Serum KIM-1/100 <sup>f</sup>	0.133	1.069 (0.980-1.167)	0.122	1.243 (0.943-1.639)
Serum REG Iα/100 <sup>d</sup>	0.001	1.737 (1.263-2.388)	0.022	1.799 (1.088-2.975)

\*: The multivariate logistic regression analysis identified the independent influencing factors for highand very-high risk patients with CKD in accordance with KDIGO risk stratification.

560 The two logistic regression analyses have been adjusted the sex, diabetes, hypertension, and FBG.

561 FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1:

562 kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration rate,

563 CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

564 a: years, b: mmol/L, c:  $\mu$ mol /L, d: ng/mL, e: mg/L, f: pg/mL.



Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum
Myoglobin in different groups.

In all participants: the CKD groups were classified in accordance with eGFR levels as described in methods. A1: Distribution of serum Cys-C. The serum Cys-C level of non-CKD group was significantly lower compared to each of the G2, G3, G4, and G5 groups individually. B1: Distribution of serum KIM-1. C1: Distribution of serum REG Ia. The serum REG Ia level of non-CKD group was significantly lower compared to each of the G1, G2, G3, G4, and G5 groups individually. D1: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower compared to each of the G3, G4, and G5 groups individually. 

In patients with CKD: the patients were classified in accordance with 2012 KDIGO risk
stratification as described in methods. A2: Distribution of serum Cys-C. B2:
Distribution of serum KIM-1. C2: Distribution of serum REG Iα. D2: Distribution of
serum Myoglobin.

17 CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes,
18 Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα.
19 \*\*: P < 0.050, \*\*\*: P < 0.001.</li>

 


Figure 2. Correlations between serum REG Ia and different markers in all participants. A: correlation between serum REG Ia and Scr (r = 0.753, P < 0.001), B: correlation between serum REG Ia and BUN (r = 0.733, P < 0.001), C: correlation between serum REG Ia and serum Cys-C (r = 0.678, P < 0.001), D: correlation between serum REG Ia and serum KIM-1 (r = 0.217, P < 0.001), E: correlation between serum REG Ia and eGFR (r = -0.789, P < 0.001). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG Ia: regenerating protein Ia.

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Figure 3. Ability of screening patients with CKD. The AUC of serum REG Ia was 0.860 30 31 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 (95% CI: 0.801-0.890), 32 while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% 33 CI: 0.581-0.699). The AUC of serum REG Ia was similar to serum Creatinine and 34 serum Cys-C (P > 0.050), and was significantly higher than serum KIM-1 and serum 35 36 Myoglobin (P < 0.001). CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Ia: regenerating protein Ia, Cys-C: cystatin C, 37 38 KIM-1: kidney injury molecular-1.

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	Supplementary mate	erial									uding f	4 on 13		
2	Supplementary data T	able 1. C	Correlatio	ons betwo	een serur	n REG Io	a and dif	ferent ma	arkers in	all partic	e sipantន្ត្ <u>ម</u> ា	Febru		
	Spearman Analyses (r)	1	2	3	4	5	6	7	8	9	10 <sup>1</sup> 0 <sup>1</sup>	Jary 11 20	12	13
	Age (1)	1.000									ed to t	25. Do		
	Sex (2)	-0.060	1.000								Superi ext and	wnload		
	Diabetes (3)	0.367***	0.080**	1.000							eur (A d data	ded fro		
	Hypertension (4)	0.262***	0.087**	0.236***	1.000						BES)	m http		
	CVD (5)	0.302***	0.024	0.125***	0.234***	1.000					g, Altr	o://bmj		
	<b>FBG (6)</b>	0.258***	0.003	0.567***	0.050	0.062	1.000				aining	open.		
	Scr (7)	0.037	0.294***	0.030	0.409***	0.098***	-0.159***	1.000			, and	bmj.cc		
	BUN (8)	0.157***	0.170***	0.136***	0.442***	0.125***	-0.072**	0.845***	1.000		simila	en la		
	UA (9)	-0.086**	0.267***	0.008	0.137***	0.036	-0.051	0.420***	0.379***	1.000	r techr	June		
	eGFR (10)	-0.245***	-0.085**	-0.081**	-0.464***	-0.148****	0.123****	-0.927***	-0.856***	-0.328***	1.000 gie	14, 20;		
	Serum Cys-C (11)	-0.073	0.048	-0.237***	0.034***	0.079	-0.251***	0.811***	0.741***	0.336***	-0.82	25 1.000 at A		
	Serum KIM-1 (12)	0.069	0.034	0.141***	0.078	0.031	0.105**	0.091**	0.121***	-0.076	-0.111**	0.111** gence	1.000	
	Serum Myoglobin (13)	0.138***	0.117***	0.098**	0.229***	0.153***	0.024	0.648***	0.606***	0.210***	-0.661***	е В. 492***	0.066	1.000

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	<b>Serum REG Ια (14)</b> 0.154***	0.059 0.073** 0.369*** 0	.099*** -0.134*** 0.753*** 0.733	;*** 0.275*** -0.78 6 6	0.217*** 0.565*** 1.000
	CVD: chronic kidney disease, FBG: fa	st blood glucose, Scr: serum cr	reatinine, BUN: blood urea nitroge	en, UA: serum uric a	: estimated glomerular filtration rate,
ŀ	Cys-C: cystatin C, KIM-1: kidney injur	y molecular-1, REG Iα: regener	rating protein Iα. **: <i>P</i> < 0.050, **	*: <i>P</i> < 0.001. tedgneme t	
5				o text:	
6	Supplementary data Table 2. Dis	tribution of serum Cys-C,	serum KIM-1, serum REG 1	i α and serum Mya a a bin i	in KDIGO risk stratification.
	KDIGO	Low Risk (18.00%)	Moderate Risk (20.70%)	퇴수 15 High Risk (17크래)	Very-high Risk
	<b>Risk Stratification Groups</b>			hing (1 hing)	(44.10%)
	Serum Cys-C (mg/L)	1.23 (1.01-1.43)	1.22 (1.05-1.45)	1.54 (1.17-179)	2.57 (1.94-3.53)
	Serum KIM-1 (pg/mL)	255.77 (211.33-279.86)	262.46 (205.21-312.24)	275.72 (227.08-377.36)	247.28 (142.72-417.99)
	Serum Myoglobin (ng/mL)	29.30 (18.97-46.25)	41.50 (22.39-51.82)	30.25 (20.60-30.52)	67.00 (51.70-113.68)
	Serum REG Ια (ng/mL)	48.86 (34.18-78.28)	76.05 (51.09-120.54)	79.18 (58.64-1 a 3.7 a)	184.38 (108.81-314.71)
7	Cys-C: cystatin C, KIM-1: kidney injur	y molecular-1, REG Ι α: regen	erating protein Ι α, KDIGO: kidn	ey disease improving	tcomes.
3	The data were presented in quartiles.			iologi	
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Page 3 1 2	3 of 34		BMJ Open by copyright, ir								
3 4 5 6	10	Supplementary d	874 on 13 ncluding fo								
7 8		-	Variables	AUC (95% CI)	Cutoff	Sensitivity	Specificity	Fee Perual Enses	NPV		
9 10				· · · ·	value	(%)	(%)	r 1 20 relat	(%)		
11 12		-	3	25. Do ement t							
13 14 15			Serum REG Ia (ng/mL)	0.860 (0.813-0.899)	70.82	71.63	86.89	ext and	46.85		
15 16			Serum Creatinine (µmol/L)	0.850 (0.801-0.890)	88.00	69.23	96.72	data (S)	47.90		
17 18 10			Serum Cys-C (mg/L)	0.842 (0.793-0.883)	1.33	72.12	91.80	a minir	49.09		
20			Serum KIM-1 (pg/mL)	0.714 (0.656-0.767) ***	232.98	63.94	68.85	ġ. <del>p</del> ≥ <sup>8</sup> ₫.93	35.09		
21 22 23			Serum Myoglobin (ng/mL)	0.642 (0.581-0.699) ***	59.70	48.08	81.97	njæen trainir	30.72		
23 24 25		-	Ability of screening hig	n and very-high risk patient	ts according t	to KDIGO risk	stratification	c C D partic	cipants		
26 27			Serum REG Ia (ng/mL)	0.769 (0.712-0.819)	76.05	82.80	62.63	d simil:	69.32		
28 29			Serum Cys-C (mg/L)	0.865 (0.817-0.904) ***	1.63	75.16	92.93	n 1921.65	70.00		
30 31			Serum KIM-1 (pg/mL)	0.528 (0.465-0.590) ***	327.10	36.94	87.88	e 14.69	46.49		
32 33	11	CKD: chronic kidne	y disease, KDIGO: kidney disea	se improving global outcome	es, Cys-C: cys	tatin C, KIM-1:	kidney injury i	rolecular-1, RI	EG I α: regene	rating protein I α.	
34 35	12	AUC: area under the receiver operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.									
36 37 38	13	Comparing with serv	um REG Iα. ***: <i>P</i> < 0.001.					nce Bibl			
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Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications 



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## Association between elevated serum REG Ia levels and eGFR decline in patients with chronic kidney disease: a cross-sectional study in eastern China

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36	Abstract
37	Objectives
38	This study conducted to demonstrate the relationship between levels of serum
39	regenerating protein Ia (REG Ia) and estimated glomerular filtration rate (eGFR) and
40	to explore the efficiency of REG Ia in chronic kidney disease (CKD) detection.
41	Design
42	A cross-sectional study.
43	Setting
44	Conducted in eastern China between August 2022 and August 2023.
45	Participants
46	880 participants which age older than 18 years were enrolled in this study, with 220
47	non-CKD participants (111 in male, 50.45%) and 660 patients with CKD (366 in male,
48	55.45%). The diagnostic criteria of CKD were in accordance with the kidney disease:
49	improving global outcomes (KDIGO) guidelines in 2012. The exclusion criteria were
50	included with involvement in other trials, acute kidney injury, patients with end-stage
51	of kidney disease who are undergoing renal replacement therapy, pregnancy, active
52	infection, gastrointestinal or pancreatic inflammation, history of gastrointestinal or
53	pancreatic resections, cancer, mental disorders.
54	Results
55	In CKD group, the levels of serum REG Ia (125.54 [60.28-303.39] ng/mL) were
56	significantly higher compared to those in non-CKD group (24.62 [14.09-37.32] ng/mL,
57	P < 0.001). Serum REG Ia exhibited a positive relationship with serum creatinine (Scr),
58	cystatin C (Cys-C), and kidney injury molecular-1(KIM-1), while showed a negative
59	relationship with eGFR. After adjusting for sex, diabetes, hypertension, and fasting
60	blood glucose (FBG), the multivariate regression analysis revealed a significant
61	association between serum REG Ia and eGFR (OR=1.737 [1.263-2.388], $P = 0.001$ ).
62	Furthermore, levels of serum REG I $\alpha$ were found to gradually increase with the decline
63	of kidney function related to eGFR ( $P < 0.001$ ). Serum REG I $\alpha$ was recognized to play
64	a role in screening CKD patients, with an area under the receiver operating

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characteristic curves (AUC) of 0.860 (0.813-0.899), providing a sensitivity of 71.63%,
a specificity of 86.89%, a positive predictive value of (PPV) 94.30%, and a negative
predictive value (NPV) of 46.85%. Additionally, serum REG Iα had an AUC of 0.769
(0.712-0.819) in screening patients at high and very-high risk for CKD according
KDIGO risk stratification, exhibiting significantly higher sensitivity compared to serum
Cys-C and KIM-1 (82.80% vs 75.16% and 36.94%).
Conclusions

This study provided evidence that levels of serum REG Iα were notably elevated in
patients with CKD and may be closely related to kidney function. The findings indicate
potential utility for REG Iα as a biomarker in CKD.

75 Clinical trial registration

The study was approved by the ethics committee of Zhongda Hospital
(2022ZDSYLL204-P01) and was in compliance with the Helsinki Declaration. The
study had a clinical study registration number of ChiCTR2300072247.

## 80 Data availability statement

The data underlying this article is available from the corresponding author underreasonable request.

## 83 Patient and public involvement statement

84 It was not appropriate or possible to involve patients or the public in the design, or85 conduct, or reporting, or dissemination plans of our research.

## 87 Strengths and limitations of this study

- We utilized different logistic regression analyses to adjust for various confounding
   factors and investigate the relationship between serum REG Iα and kidney function.
- This study was the first time to apply KDIGO risk stratification in CKD population,
   analyzing the potential association between serum REG Iα and the risk of CKD
   progression.
- We applied the DeLong test to statistically analyze the differences in AUC values

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2 3	04	among the higher of CVD accessing their diagnostic shiliting
4 5	94	among the biomarkers of CKD, assessing their diagnostic abilities.
6 7	95	
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29 30	107	stratification;
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List of abbreviations	
Regenerating protein Ia	REG Ia
Diabetic kidney disease	DKD
Chronic kidney disease	CKD
Receiver operating characteristic curves	ROC
Cystatin C	Cys-C
Kidney injury molecular-1	KIM-1
Area under the ROC	AUC
End stage kidney disease	ESKD
Serum creatinine	Scr
Blood urea nitrogen	BUN
Estimate glomerular filtration rate	eGFR
Urine albuminuria creatine ratio	UACR
Kidney disease improving global outcomes	KDIGO
Pancreatic stone protein	PSP
Chronic kidney disease epidemiology collaboration	CKD-EPI
Unilateral ureteral obstruction	UUO
Uric acid	UA
Fast blood glucose	FBG
Cardiovascular disease	CVD
Standard deviation	SD

## 111 Introduction

112 Chronic kidney disease (CKD) has a broad range of underlying etiologies and variable 113 progression rates, <sup>1,2</sup> and may become the fifth leading cause of death worldwide by 114 2040.<sup>3</sup> The endpoint of CKD is end stage kidney disease (ESKD) in which 90% of 115 kidney function reduced, making it impossible to sustain life over the long term.<sup>2</sup> The 116 high prevalence, low detection rate, severe outcomes, and substantial medical costs of 117 CKD make it a significant global health concern.<sup>4</sup> Early prevention, detection, and 118 treatment can lead to better outcomes and prevent ESKD progression.

Biomarkers such as serum creatinine (Scr), blood urea nitrogen (BUN), estimate glomerular filtration rate (eGFR), and urine albuminuria creatine ratio (UACR) are routinely used to assess the severity of CKD.<sup>5-8</sup> In 2012, Kidney Disease: Improving Global Outcomes (KDIGO) has taken the eGFR and UACR to create risk stratification strategies, assisting in the tailored management of CKD and guiding treatment for those at a higher risk of progression.<sup>9</sup> New biomarkers like, cystatin C (Cys-C), kidney injury molecular-1 (KIM-1), and β2-microglobuline have shown great application potential in enhancing the precision of early-stage CKD screening, either independently or in conjunction.<sup>2,3,10,11</sup> However, most biomarkers failed to match clinical expectations.<sup>12-</sup> <sup>15</sup> Few biomarkers can both detect and assess risk stratification effectively in patients with CKD. Thus, discovering a biomarker can screen kidney function decline and evaluate CKD progression risk is crucial.

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Regenerating protein I $\alpha$  (REG I $\alpha$ ) is a 16 kDa protein primarily secreted by the pancreas and intestine.<sup>16</sup> also known as pancreatic stone protein (PSP).<sup>17</sup> The involvement of REG Ia plays a role in the processes of cellular proliferation and regeneration.<sup>18,19</sup> Recent researchers have identified the presence of REG Ia in patients with various kidney diseases.<sup>20,21</sup> Our previous studies also have indicated that serum levels of REG Iα elevate in patients with diabetic kidney disease (DKD), which are concordant with the finding of H. Sobajima.<sup>22-24</sup> These evidences indicate that REG Iα may serve as a biomarker of kidney insufficiency. 

139 In this study, we analyzed the association between serum REG I $\alpha$  levels and kidney

function, and further assessed its potential as a screening tool for CKD and as a biomarker of kidney function. 

#### **Study subjects**

The participants were enrolled from Zhongda Hospital between August 2022 and August 2023. The study was approved by the ethics committee (2022ZDSYLL204-P01), with a clinical study registration of ChiCTR2300072247. Informed consent was acquired from all participants.

The inclusion criteria were as follows: (1) non-CKD participant: age older than 18 years; (2) CKD patients: age older than 18 years and diagnosed with CKD in accordance with the guidelines in 2012.<sup>1</sup> The exclusion criteria were as follows: (1) enrolled in another trial; (2) acute kidney injury; (3) patients with ESKD who are undergoing renal replacement therapy; (4) pregnancy; (5) active infection; (6) acute or chronic inflammation of the gastrointestinal system and pancreas; (7) history of gastrointestinal or pancreatic resections; (8) cancer; (9) mental disorders. 

eGFR was calculated in accordance with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.<sup>25</sup> CKD groups was classified using the method proposed by the U.S. National Kidney Foundation. The risk stratification of CKD progression was defined in accordance with KDIGO risk stratification guideline.<sup>26,27</sup> The subgroup analyses was carried out in patients with CKD to explore the relationship between serum REG Ia and different degrees of kidney function impairment. 

Data collection and quality assessment

Demographics information was collected at baseline through questionnaires. All participants have undergone 12-h fasting and taken about 3mL of peripheral blood sample. The blood samples were centrifuged directly for 15 min at a rotating speed of 3,500 rpm, and the upper serum was sucked up within 6-h. The serum was immediately frozen in refrigerator (-80°C). Clinical biochemical parameters were extracted from the clinical laboratory of Hospital, such as Scr, BUN, uric acid (UA), Cys-C, Myoglobin, UACR, and fast blood glucose (FBG). The laboratory implements internal and external 

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quality control procedures directed by a Chinese Quality Control Laboratory. The
serum REG Iα levels were determined by a double antibody sandwich ELISA, as
previously described.<sup>28</sup> Serum KIM-1 was detected by an ELISA kit (KE00136) from
Proteintech.

## 173 Statistical analysis

Statistical analyses were conducted using SPSS 20.0, Med-Calc, and GraphPad Prism 8.0. Continuous data with normal distribution were summarized as mean  $\pm$  standard deviation (SD), otherwise as median with interquartile range. For categorical variables, the frequency with a percentage of each category was calculated. Spearman's rank correlation analyses and ordinal logistic regression was used to measure the associations between serum REG Ia and other biomarkers of kidney function. Multivariate logistic regression analysis was utilized to identify the independent factors of kidney dysfunction. The area under the receiver operating characteristic curve (AUC) was plotted to analyze the ability of serum Cys-C, KIM-1, and REG Ia to screen the patients with CKD, and detect the high and very-high risk patients. The study assessed evaluate accuracy using sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy, and applied the DeLong test to statistically analyze AUC differences between receiver operating characteristic curves. *P* value of <0.050 using two-tailed tests was considered statistically significant. 

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188 Results

## 189 Baseline characteristics of the study population

Overall, 880 participants were finally enrolled, with 220 non-CKD participants and 660 patients with CKD (Supplementary data Figure 1). Differences in age, complication diseases (diabetes and hypertension), kidney function biomarkers (Scr, BUN, UA, eGFR, Cys-C, and KIM-1), and serum myoglobin were observed between patients with CKD and non-CKD participants (all P < 0.001, Table 1). Serum REG Ia levels were notably higher in patients with CKD (125.54 [60.28-303.39] ng/mL), than in non-CKD participants (24.62[14.09-37.32] ng/mL, P < 0.001, Table 1). Serum Cys-C, REG Ia, and myoglobin showed increasing trends as eGFR levels decreased (Figure 1: A1, C1,

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and D1). A significant difference in serum KIM-1 was found between the non-CKD group and CKD groups (G2 to G5, P < 0.010, Figure 1: B1). Two reanalyzes balanced the effects of CVD on the distribution of serum REG Ia and myoglobin (supplementary data Figure 2), and the effects of diabetes on patients with CKD (Supplementary data Figure 3).

#### Relationship between serum REG Ia and kidney function

This study explored the relationship between serum REG Ia levels and kidney function biomarkers. In figure 2, Spearman correlation analyses demonstrated that serum REG Ia was positively correlated with Scr (r = 0.753, P < 0.001), BUN (r = 0.733, P < 0.001), serum Cvs-C (r = 0.678, P < 0.001), and serum KIM-1 (r = 0.217, P < 0.001), and negatively correlated with eGFR (r = -0.789, P < 0.001). The comprehensive correlations between serum REG Ia and various markers were presented in supplementary data Table 1. In ordinal logistic regression analysis carried out in all participants, eGFR values were used as a grade-dependent variable in the model. The results showed that serum REG Ia/100 levels was markedly associated with eGFR  $(OR=1.737\ 95\%\ CI:\ 1.263-2.388,\ P=0.001,\ Table\ 2).$ 

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## Subgroup analysis in patients with CKD

To investigate the relationship between serum REG I $\alpha$  and the risk of CKD progression, 256 CKD patients were included in the sub-research and were classified by eGFR and UACR levels in accordance with KDIGO risk stratification guideline.<sup>1</sup> The patients were divided into four groups to assess the progression and prognostic, namely, low risk (18.00%), moderate risk (20.70%), high risk (17.20%) and very-high risk (44.10%, supplementary data Table 2). We found that levels of serum Cys-C significantly increase to 2.57 (1.94, 3.53) mg/L in the very-high risk group (P < 0.001, Figure 1: A2). In the same trends, the levels of serum REG Ia significantly increase to 184.38 (108.81-314.71) ng/mL in the very-high risk group (P < 0.001, Figure 1: C2). The very-high risk group had the highest serum myoglobin level among the four groups (P < 0.001, Figure 1: D2). The multiple logistic regression analysis carried out in patients with CKD, demonstrated that serum REG  $I\alpha/100$  was an independent influencing factor for patients

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with CKD who are at high and very-high risk (OR=1.799, 95% CI: 1.088-2.975, P =
0.022, Table 2).

## 229 Ability of serum REG Ia in screening patients with kidney dysfunction

In evaluating the potential application of serum REG I $\alpha$  as a screening tool for patients with CKD and its ability to differentiate the risk stratification, we performed receiver operating characteristic curve (ROC) analyses (Figure 3 and supplementary data Table 3). The AUC for serum REG Ia to detect patients with CKD was 0.860 (95% CI: 0.813-0.899) as well as serum creatinine (0.850, 95% CI: 0.801-0.890) and Cys-C (0.842, 95% CI: 0.793-0.883), with a cutoff value of 70.82 ng/mL, sensitivity of 71.63%, specificity of 86.89%, positive predictive value (PPV) of 94.30%, and negative predictive value (NPV) of 46.85%. By contrast, serum KIM-1 had a lower AUC than serum REG Ia, measuring 0.714 (95% CI: 0.656-0.767, P < 0.001). Serum myoglobin had the lowest AUC among the five biomarkers, measuring 0.642 (95% CI: 0.581-0.699, P < 0.001). Furthermore, we analyzed the ability of serum Cys-C, KIM-1, and REG Ia in distinguishing high and very-high risk patients with CKD in accordance with the KDIGO risk stratification. As shown in supplementary data Table 3, serum Cys-C had an AUC of 0.865 (95% CI: 0.817-0.904, P < 0.010), which was the highest AUC among the three markers. Serum REG Ia had an AUC of 0.769 (95% CI: 0.712-0.819), showing better performance than serum KIM-1 (P < 0.010). The cutoff value of serum REG Ia was 76.05 ng/mL, and the sensitivity, specificity, PPV, and NPV were 82.80%, 62.63%, 77.38%, and 69.32%, respectively. Notably, serum REG Iα had a significantly higher sensitivity than serum Cys-C and KIM-1. 

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249 Discussion

This study was the first time to investigate the whole CKD population, and confirm the
upregulation of serum REG Iα in patients with CKD. Initially, the serum levels of REG
Iα showed a gradually increasing trend with the decrease of eGFR. Second, serum REG
Iα was negatively correlated with eGFR and positively correlated with Scr, BUN, serum
Cys-C, and serum KIM-1 levels. Third, serum REG Iα was an independent risk factor
for high and very-high risk patients in accordance with the KDIGO risk stratification.

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Finally, serum REG Iα played an important role in screening patients with CKD anddetecting high and very-high risk patients in CKD.

REG Ia was a low molecular weight (16 kDa) protein initially discovered in pancreas, where it exhibited a remarkable ability to inhibit the formation of calcium carbonate stones in pancreatic ducts.<sup>29</sup> Hence, REG Ia was also called pancreatic stone protein (PSP) at the time.<sup>30</sup> Immunocytochemical analyses indicated that REG Ia protein was overexpressed in impaired kidney, with predominant localization observed in proximal tubules and thick ascending limbs of Henle's loops.<sup>20</sup> Then, some studies declared that urine PSP (REG Ia) and REG Ia gene might indicate kidney tubular dysfunction and potentially serve as an early biomarker of DKD detection and progression.<sup>22,31,32</sup> Our previous studies provided compelling evidence, that serum REG Iα levels were notably elevated in patients with type 2 diabetes mellitus who had kidney dysfunction. And we also observed that pregnant women with early renal dysfunction exhibited similar elevations in serum REG Ia levels.<sup>24,33</sup> Notably, the serum REG Ia levels remained significantly elevated when the scope of this study was expanded to the entire CKD population.

We incorporated myoglobin (17.6 kDa)<sup>34</sup> to investigate whether the elevated level of serum REG Ia represents a universal phenomenon of accumulation in CKD. The myoglobin was identified as a biomarker for acute myocardial ischemia and rhabdomyolysis.<sup>35</sup> Some studies indicated that serum myoglobin levels increase in kidney impairment. This accumulation was primarily attributed to slower clearance in CKD.<sup>36</sup> We showed that serum levels of myoglobin have no differences between non-CKD participants and patients with early stage of CKD (group G1 and group G2). And there was a gradual increase in patients with medium to end-stages of CKD (group G3 to group G5). The possible reason was as follows: in the early stages of kidney injury, compensatory glomerular hypertrophy with hyperfiltration state was known to exist. This mechanism allowed some low molecular weight protein to be filtered through the glomerular basement membrane, resulting in no discernible difference during the early stage of CKD (eGFR  $\geq$  60 mL/min per 1.73 m<sup>2</sup>), and even appearing as a transient

decrease. With progression to medium to end-stages of CKD (eGFR  $\leq 60$  mL/min per 1.73 m<sup>2</sup>), the glomerular basement membrane thickened and led to significant decline in glomerular filtration function, ultimately causing proteins accumulation in serum.<sup>37</sup> The serum levels of REG Ia showed a gradual increase in medium to end-stages of CKD, which was similar to the rise observed in serum myoglobin levels. However, it exhibited a significant increase in the early stage of CKD, which was different with serum myoglobin. This suggested that, in addition to the sensitive accumulation of serum REG I $\alpha$  as a low molecular weight protein, there might be a specific production when it comes to kidney impairment.

The etiology underlying the upregulation of REG Ia production in patients with CKD remains elusive. Two potential mechanisms are considered as follows. First, REG Ia resists apoptosis and promotes cell proliferation in different inflammation situations.<sup>38-</sup> <sup>42</sup> Studies have shown that cytokines such as IL-6 can increase the proliferation of REG Iα, which is involved in cell regeneration and repair.<sup>42,43</sup> In CKD, the occurrence and progression of the disease are closely related to the expression of multiple inflammatory cytokines, including IL-1, IL-6, and TNF-a. <sup>37,44,45</sup> Under the stimulation of chronic inflammation, different types of renal cells secrete REG Ia locally to participate in kidney anti-apoptosis and proliferation and against kidney fibrosis in the development of CKD. The secreted REG Ia enters the circulation through reabsorption by kidney tubules, resulting in a significant increase in serum REG Ia levels. Thus, REG Ia might serve as an inflammatory factor involved in kidney diseases. Second, REG Ia is primarily synthesized in the pancreas and released into the circulation.<sup>18,46</sup> A hypothesis suggests that a cross-talk mechanism exists between the pancreas and kidney when renal damage occurs. This interaction may prompt the pancreas to produce more REG I $\alpha$  in response to kidney injury, resulting the elevation in serum. Although the exact cause of REG Ia upregulation remains unclear, these two proposed mechanisms provided potential insights into the relationship between REG Ia and CKD. 

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At present, the assessment of CKD generally focused on glomerular filtration capacity,
which is characterized by Scr, UACR, and eGFR.<sup>2</sup> The stabilities of these factors are

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compromised by age, dietary intake, and physical activity. In general, the performance of eGFR is poor for populations outside of North America, Australia, and Europe, and accuracy decreases at the extremes in the distribution of age, and body composition.<sup>7</sup> Therefore, many new biomarkers of kidney impairment have been discovered. Cys-C is produced by all nucleated cells at a steady rate and freely filtered by the kidney.<sup>47</sup> The accumulation of its levels is observed in case of glomerular filtration dysfunction, with limited impact and strong stability.<sup>48</sup> Eventually, serum Cys-C is incorporated into the eGFR equation as a reliable and sensitive indicator of kidney function. The sensitivity of serum Cys-C is heightened in the early stages of CKD compared to creatinine.<sup>49,50</sup> Another biomarker is KIM-1,<sup>51,52</sup> which is secreted following kidney proximal tubular injury, and has emerged as a sensitive and specific biomarker of acute kidney injury and CKD progression.53-56 In this study, serum REG Ia strongly correlated with serum creatinine and Cys-C, and had a similar performance to serum creatinine and Cys-C in detecting patients with CKD. The serum levels of REG Ia were more likely related to glomerular filtration capacity according to the correlation analyses, although the mechanism of its production and degradation or excretion in CKD remained unclear. We indicated that serum REG I $\alpha$  might be more sensitive than serum creatinine and Cys-C in detecting ultra-early stage of kidney dysfunction. Similar phenomenon was observed in distribution analyses in KDIGO risk stratification. The KDIGO risk classification was widely used in evaluating the progression and prognosis risk in patients with CKD.<sup>57-60</sup> Serum levels of REG Ia gradually elevated with higher KDIGO risk stratification categories. And it emerged as an independent risk factor for patients with CKD categorized as high and very-high risk. Our findings provided a new insight that serum REG Ia performed better than serum KIM-1 in screening patients with CKD and detecting high and very-high risk patients with CKD. Moreover, serum REG Ia displayed a highest sensitivity in identifying high and very-high risk patients with CKD. These results highlight the potential application of serum REG I $\alpha$  as a valuable biomarker in the screening of patients with CKD and the assessment of CKD risk.

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We observed that patients with CKD were older than non-CKD participants, and they had higher rates of diabetes and hypertension. This finding was consistent with the typical etiology of CKD. Globally, diabetes and hypertension are recognized as the primary causes of CKD.<sup>2</sup> Diabetes accounts for 30-50% of all CKD cases and affects approximately 285 million adults worldwide. A consistent finding in observational studies shows that the increased risk of developing is associated with blood pressure control. These findings were consistent with the expected proportion of CKD etiology in our study.

There are some limitations in this study. First, it is a cross-sectional assessment and further follow-up studies must be conducted to provide a more comprehensive understanding. Second, although robust biomarkers such as creatinine and Cys-C are already available for the diagnosis of CKD, the potential efficacy of REG I $\alpha$  as a combined biomarker is anticipated to be revealed in subsequent large-scale analyses. Third, this study did not definitively identify the exact source of elevated REG Ia in patients with CKD. Therefore, further mechanistic studies should be conducted to investigate the origins of REG I $\alpha$  in the situation of kidney impairment. Forth, to address residual confounding, future studies should incorporate additional covariates such as drug use and different causes of CKD and conduct sensitivity analyses to assess potential biases. Moreover, to enhance causality and generalizability, longitudinal designs with larger sample sizes and diverse populations should be considered in forthcoming research. 

## 364 Conclusion

This study provided compelling evidence that serum REG Iα was significantly
upregulated in the patients with CKD and strongly associated with kidney function.
Serum REG Iα emerged as a significant biomarker for detecting kidney function decline.

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## 369 Contributorship statement

Professor Ling Li acted as guarantor. Ling Li conceptualized the study; Bin Wang reviewed and edited the manuscript; Nan Huang and Xiangyun Zhu were responsible for the data analysis and wrote the original draft. Nan Huang, Sheng Chen, Xiaodong Wu, Hui Wang, Zhiyi Shu and Xi Huang were responsible for inclusion of population, collection of samples and data, and the experiments. Jianling Bai and Jinfang Sun were responsible for guidance on data analysis. Pingsheng Chen and Xiuxiu Hu were responsible for analysis of kidney biopsy tissues from CKD patients. Rolf Graf provided the excellent technical support for this study.

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Tables	
Table 1. Clinical characteristics of study population at baseline examination.	
CKD: chronic kidney disease; The data were presented in quartiles.	
Table 2. The logistic regression analyses showing the relationship between variables	
and kidney function.	
#: The ordinal multiple logistic regression showing variables independently associated with eGFR levels	
in all participants. *: The multivariate logistic regression analysis identified the independent influencing	
factors for high and very-high risk patients with CKD in accordance with KDIGO risk stratification.	
The two logistic regression analyses have been adjusted the sex, diabetes, hypertension, and FBG.	
FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1:	
kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration rate,	
CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.	
a: years, b: mmol/L, c: µmol /L, d: ng/mL, e: mg/L, f: pg/mL.	
Figures	
Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG I $\alpha$ , and serum	
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A1, B1, C1, and D1 are in all participants: the CKD groups were classified in accordance with eGFR	
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in patients with CKD: the patients were classified in accordance with 2012 KDIGO risk stratification	
as described in methods. A2: Distribution of serum Cys-C. B2: Distribution of serum KIM-1. C2:	
Distribution of serum REG Ia. D2: Distribution of serum Myoglobin.	
CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin	
C, KIM-1: kidney injury molecular-1, REG Ia: regenerating protein Ia. *: $P < 0.050$ , **: $P < 0.010$ ,	
***: <i>P</i> < 0.001.	
Figure 2. Correlations between serum REG I $\alpha$ and different markers in all participants.	
A: correlation between serum REG Ia and Scr (r = 0.753, $P < 0.001$ ), B: correlation between serum 23	

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REG Ia and BUN (r = 0.733, P < 0.001), C: correlation between serum REG Ia and serum Cys-C r = 0.678, P < 0.001), D: correlation between serum REG Ia and serum KIM-1 (r = 0.217, P < 0.001) .001), E: correlation between serum REG Ia and eGFR (r = -0.789, P < 0.001). Scr: serum reatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: stimated glomerular filtration rate, REG Ia: regenerating protein Ia.

- igure 3. Ability of screening patients with CKD.
- The AUC of serum REG Iα was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was .850 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum IM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 95% CI: 0.581-0.699). The AUC of serum REG I $\alpha$  was similar to serum Creatinine and serum Cys-C(P > 0.050), and was significantly higher than serum KIM-1 and serum Myoglobin (P < 0.001). CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Ia:
- egenerating protein Iα, Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

## upplementary material

upplementary data Table 1. Correlations between serum REG Ia and different markers n all participants.

- VD: chronic kidney disease, FBG: fast blood glucose, Scr: serum creatinine, BUN: blood urea nitrogen,
- A: serum uric acid, eGFR: estimated glomerular filtration rate, Cys-C: cystatin C, KIM-1: kidney injury
- nolecular-1, REG Iα: regenerating protein Iα. \*\*: P<0.050, \*\*\*: P<0.001.
  - upplementary data Table 2. Distribution of serum Cys-C, serum KIM-1, serum REG
- I  $\alpha$  and serum Myoglobin in KDIGO risk stratification.
- $\Delta x$ -Cystatin C, KIM-1: kidney injury molecular-1, REG I  $\alpha$ : regenerating protein I  $\alpha$ , KDIGO:
  - idney disease improving global outcomes. The data were presented in quartiles.
- upplementary data Table 3. Ability of different biomarkers to screen patients with
- CKD.

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3 4	615	CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C,				
5 6	616	KIM-1: kidney injury molecular-1, REG I $\alpha$ : regenerating protein I $\alpha$ . AUC: area under the receiver				
7 8	617	operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.				
9 10 11	618 Comparing with serum REG Ia. ***: $P < 0.001$ .					
12 13	619					
13 14 15	620	Supplementary data Figure 1. Flowchart of participant selection.				
15 16 17 18	621	In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion				
	622	criterion, participants were excluded because of missing critical data (n=108), active infection (n=12),				
19 20	623	acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental				
21	624	disorders (n=6). CKD: chronic kidney disease.				
23 24	625					
25 26 27 28 29 30 31 32	626	Supplementary data Figure 2. Distribution of serum REG Ia and Myoglobin in non-				
	627	CKD and different CKD groups, which participants without CVD.				
	628	A: Distribution of serum REG Ia. The serum REG Ia level of non-CKD group was significantly				
	629	lower than that of G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin.				
33 34	630	The serum myoglobin level of non-CKD was significantly lower than that of G4 and G5 groups				
35 36	631	individually. CKD: chronic kidney disease, CVD: cardiovascular disease, REG Iα: regenerating				
37 38	632	protein Ia. *: $P < 0.050$ , **: $P < 0.010$ , ***: $P < 0.001$ .				
39 40	633					
41 42	634	Supplementary data Figure 3. Levels of serum REG I $\alpha$ in CKD patients with and				
43 44	635	without T2DM.				
45 46	636	The median with interquartile range of serum REG Ia was 144.37 (54.71-357.59) ng/ml in CKD patients				
47 48	637	without T2DM, and 116.57 (65.86-276.34) ng/ml. REG Ia: regenerating protein Ia, T2DM: type 2				
49 50	638	diabetes mellitus.				
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# Tables

Table 1. Clinical characteristics of study population at baseline examination.

	Non-CKD Participants	<b>CKD</b> Patients	P Value	
Number	220	660	-	
DEMOGRAPHICS				
Age (years)	53 (40-62)	62 (50-72)	< 0.001	
Sex (male, %)	111 (50.45)	366 (55.45)	0.212	
Smoking (yes, %)	41 (18.64)	142 (21.52)	0.141	
Drinking (yes, %)	30 (13.64)	84 (12.73)	0.823	
COMPLICATION DISEASES				
Diabetes (yes, %)	75 (34.09)	350 (53.03)	< 0.001	
Hypertension (yes, %)	65 (29.55)	501 (75.91)	< 0.001	
Cardiovascular disease (CVD, yes, %)	44 (20.00)	149 (22.58)	0.453	
LABORATORY MEASUREMENTS				
Fast blood glucose (FBG, mmol/L)	5.45 (4.99-6.67)	5.34 (4.57-7.11)	0.008	
Serum creatinine (Scr, µmol/L)	64.00 (55.25-76.00)	126.00 (83.00-418.50)	< 0.001	
Blood urea nitrogen (BUN, mmol/L)	5.20 (4.30-6.28)	10.30 (7.00-18.60)	< 0.001	
Serum uric acid (UA, µmol/L)	301.00 (256.00-365.25)	354.00 (290.00-443.00)	< 0.001	
Estimated glomerular filtration rate	102 37 (05 73 112 36)	44.03 (10.06.77.00)	< 0.001	
(eGFR, mL/min per 1.73 m <sup>2</sup> )	102.57 (95.75-112.50)	44.03 (10.90-11.09)	< 0.001	
Serum cystatin C (Cys-C, mg/L)	1.10 (0.99-1.24)	1.79 (1.23-3.50)	< 0.001	
Serum kidney injury molecular-1	186.28 (57.22-266.88)	247.72 (175.10-334.13)	< 0.001	
(KIM-1, pg/mL)	100120 (01122 200100)	(1,0110 00 1110)		
Serum Myoglobin (ng/mL)	46.81 (33.49-58.00)	64.04 (35.96-112.51)	< 0.001	
Serum regenerating protein Ia	24.62 (14.09-37.32)	125.54 (60.28-303.39)	< 0.001	
(REG Ia, ng/mL)				

CKD: chronic kidney disease; The data were presented in quartiles.

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Table 2. The logistic regression analyses showing the relationship between variables and kidney function.

	Ordinal Logistic Regression <sup>#</sup>		Multivariate Logistic Regression*	
	P Value	OR (95% CI)	P Value	OR (95% CI)
Age <sup>a</sup>	0.050	1.020 (1.001-1.041)	0.009	0.966 (0.942-0.992)
BUN <sup>b</sup>	< 0.001	1.266 (1.165-1.376)	< 0.001	1.440 (1.216-1.706)
UA°	0.337	1.001 (0.999-1.004)	0.085	1.003 (1.000-1.007)
Serum Myoglobin <sup>d</sup>	0.165	1.005 (0.998-1.013)	N/A	N/A
Serum Cys-C <sup>e</sup>	< 0.001	6.784 (4.016-11.460)	0.071	1.853 (0.949-3.620)
Serum KIM-1/100 <sup>f</sup>	0.133	1.069 (0.980-1.167)	0.122	1.243 (0.943-1.639)
Serum REG Ia/100 <sup>d</sup>	0.001	1.737 (1.263-2.388)	0.022	1.799 (1.088-2.975)

#: The ordinal multiple logistic regression showing variables independently associated with eGFR levels in all participants.

\*: The multivariate logistic regression analysis identified the independent influencing factors for high and very-high risk patients with CKD in accordance with KDIGO risk stratification.

The two logistic regression analyses have been adjusted the sex, diabetes, hypertension, and FBG.

FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1:

kidney injury molecular-1, REG Ia: regenerating protein Ia, eGFR: estimated glomerular filtration rate,

CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

a: years, b: mmol/L, c: µmol /L, d: ng/mL, e: mg/L, f: pg/mL.





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Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum Myoglobin in different groups.

A1, B1, C1, and D1 are in all participants: the CKD groups were classified in accordance with eGFR
levels as described in methods. A1: Distribution of serum Cys-C. B1: Distribution of serum KIM1. C1: Distribution of serum REG Iα. D1: Distribution of serum myoglobin.

A2, B2, C2, and D2 are in patients with CKD: the patients were classified in accordance with 2012 KDIGO risk stratification as described in methods. A2: Distribution of serum Cys-C. B2: Distribution of serum KIM-1. C2: Distribution of serum REG Iα. D2: Distribution of serum Myoglobin.

CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG I $\alpha$ : regenerating protein I $\alpha$ . \*: P < 0.050, \*\*: P < 0.010, \*\*\*: P < 0.001.
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Figure 2. Correlations between serum REG I $\alpha$  and different markers in all participants. A: correlation between serum REG I $\alpha$  and Scr (r = 0.753, P < 0.001), B: correlation between serum REG I $\alpha$  and serum Cys-C (r = 0.678, P < 0.001), D: correlation between serum REG I $\alpha$  and serum KIM-1 (r = 0.217, P < 0.001), E: correlation between serum REG I $\alpha$  and eGFR (r = -0.789, P < 0.001). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG I $\alpha$ : regenerating protein I $\alpha$ .

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100 - Specificity

Serum REG Ia, AUC = 0.860 (0.813-0.899);

Serum KIM-1, AUC = 0.714 (0.656-0.767):

Serum Creatinine, AUC = 0.850 (0.801-0.890); Serum Cys-C, AUC = 0.842 (0.793-0.883);

Serum Myoglobin, AUC = 0.642 (0.581-0.699);

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Sensitivity



Figure 3. Ability of screening patients with CKD. The AUC of serum REG Ia was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% CI: 0.581-0.699). The AUC of serum REG Ia was similar to serum Creatinine and serum Cys-C (P > 0.050), and was significantly higher than serum KIM-1 and serum Myoglobin (P < 0.001). CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Ia: regenerating protein Ia, Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

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Diabetes (3)	0.367***	0.080**	1.000							nd dat	aded f			
Hypertension (4)	0.262***	0.087**	0.236***	1.000						a mini				
CVD (5)	0.302***	0.024	0.125***	0.234***	1.000					ng, Al	ttp://br			
FBG (6)	0.258***	0.003	0.567***	0.050	0.062	1.000				trainir	njoper			
Scr (7)	0.037	0.294***	0.030	0.409***	0.098***	-0.159***	1.000			ıg, an	n.bmj.			
BUN (8)	0.157***	0.170***	0.136***	0.442***	0.125***	-0.072**	0.845***	1.000		d simil	com/ o			
UA (9)	-0.086**	0.267***	0.008	0.137***	0.036	-0.051	0.420***	0.379***	1.000	lar tec	on Jun			
eGFR (10)	-0.245***	-0.085**	-0.081**	-0.464***	-0.148***	0.123***	-0.927***	-0.856***	-0.328***	1.00 <b>0</b> 0	e 14, 2			
Serum Cys-C (11)	-0.073	0.048	-0.237***	0.034***	0.079	-0.251***	0.811***	0.741***	0.336***	-0.82 <b>¢/</b> **	025 1.000			
Serum KIM-1 (12)	0.069	0.034	0.141***	0.078	0.031	0.105**	0.091**	0.121***	-0.076	-0.111**	Agen 0.111**	1.000		
Serum Myoglobin (13)	0.138***	0 117***	0.098**	0 229***	0 1 5 3***	0.024	0 648***	0.606***	0 210***	-0.661***	<b>Ce</b> 0 492***	0.066	1.000	

of 41			BMJ Open	jopen-202 by copyri	
	Serum REG Ia (14) 0 154*** (	0.059 0.073** 0.369*** (	0.099*** -0.134*** 0.753*** 0	<b>3ht</b> <b>1086874</b> <b>1067</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b> <b>110</b>	8*** 0.217*** 0.565***
з	CVD: chronic kidney disease FBG: fast	blood glucose Scr. serum cre	atinine BUN: blood urea nitroge	n UA: serum uric acid <b>G</b>	timated glomerular filtration
0	C VD. enfolie kluley disease, i DG. ias	blood glucose, ser serum ered		reicy	innated giomerular mitation
4 5	C: cystatin C, KIM-1: kidney injury mo	lecular-1, REG Iα: regeneratin	g protein Iα. **: <i>P</i> < 0.050, ***:	<i>P</i> < 0.001. Interview of the second	
6	Supplementary data Table 2. Dist	tribution of serum Cys-C,	serum KIM-1, serum REG	I α and serum Mya a doin	in KDIGO risk stratific
	KDIGO Risk Stratification Groups	Low Risk (18.00%)	Moderate Risk (20.70%)	High Risk (17 miles)	Very-high Risk (44.10%)
	Serum Cys-C (mg/L)	1.23 (1.01-1.43)	1.22 (1.05-1.45)		2.57 (1.94-3.53)
	Serum KIM-1 (pg/mL)	255.77 (211.33-279.86)	262.46 (205.21-312.24)	275.72 (227.08-377.36)	247.28 (142.72-417.99
	Serum Myoglobin (ng/mL)	29.30 (18.97-46.25)	41.50 (22.39-51.82)	30.25 (20.60-30).52	67.00 (51.70-113.68)
	Serum REG Ια (ng/mL)	48.86 (34.18-78.28)	76.05 (51.09-120.54)	79.18 (58.64-1 3.7 9)	184.38 (108.81-314.71
7	Cys-C: cystatin C, KIM-1: kidney injury	y molecular-1, REG Ια: reger	nerating protein Ι α, KDIGO: ki	dney disease improving	itcomes.
8	The data were presented in quartiles.			ologi	
9				25 at es.	
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0 S	upplementary data Table 3. Ability of diffe	erent biomarkers to scre	en patients	with CKD.		74 on 13 Sluding fo			
	Variables	AUC (95% CD	Cutoff	Sensitivity	Specificity	r uses	NPV		
			value	(%)	(%)	r€20 relat	(%)		
	4	Ability of screening pa	tients with C	KD in all parti	cipants	25. Do ement			
	Serum REG Iα (ng/mL)	0.860 (0.813-0.899)	70.82	71.63	86.89	ext an	46.85		
	Serum Creatinine (µmol/L)	0.850 (0.801-0.890)	88.00	69.23	96.72	def.33 eur (A data	47.90		
	Serum Cys-C (mg/L)	0.842 (0.793-0.883)	1.33	72.12	91.80	minin	49.09		
	Serum KIM-1 (pg/mL)	0.714 (0.656-0.767) ***	232.98	63.94	68.85	<b>b</b> , <b>A i t</b>	35.09		
	Serum Myoglobin (ng/mL)	0.642 (0.581-0.699) ***	59.70	48.08	81.97	rainin	30.72		
	Ability of screening high	and very-high risk patien	ts according	to KDIGO risk	stratification	in CED parti	cipants		
	Serum REG Ia (ng/mL)	0.769 (0.712-0.819)	76.05	82.80	62.63	simila 0	69.32		
	Serum Cys-C (mg/L)	0.865 (0.817-0.904) ***	1.63	75.16	92.93	n 950.65 Ar tech	70.00		
	Serum KIM-1 (pg/mL)	0.528 (0.465-0.590) ***	327.10	36.94	87.88	molog	46.49		
1 C	KD: chronic kidney disease, KDIGO: kidney disea	se improving global outcome	es, Cys-C: cys	statin C, KIM-1	kidney injury	nerolecular-1, R	EG I α: regenerating		
2 A	UC: area under the receiver operating characteristic	area under the receiver operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.							
<b>3</b> Co	Comparing with serum REG Ia. ***: $P < 0.001$ .								

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Supplementary data Figure 1. Flowchart of participant selection. In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental disorders (n=6). CKD: chronic kidney disease.

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Supplementary data Figure 2. Distribution of serum REG I $\alpha$  and Myoglobin in non-CKD and different CKD groups, which participants without CVD. A: Distribution of serum REG I $\alpha$ . The serum REG I $\alpha$  level of non-CKD group was significantly lower than that of G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower than that of G4 and G5 groups individually. CKD: chronic kidney disease, CVD: cardiovascular disease, REG I $\alpha$ : regenerating protein I $\alpha$ . \*: *P* < 0.050, \*\*: *P* < 0.010, \*\*\*: *P* < 0.001.



Supplementary data Figure 3. Levels of serum REG Iα in CKD patients with and without T2DM. The median with interquartile range of serum REG Iα was 144.37 (54.71-357.59) ng/ml in CKD patients without T2DM, and 116.57 (65.86-276.34) ng/ml. REG Iα: regenerating protein Iα, T2DM: type 2 diabetes mellitus.

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# Association between elevated serum REG Ia levels and eGFR decline in patients with chronic kidney disease: a cross-sectional study in eastern China

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Abstract

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**Objectives** This study aimed to investigate the relationship between serum regenerating protein I $\alpha$ 36 (REG I $\alpha$ ) levels and estimated glomerular filtration rate (eGFR) and to evaluate the 37 diagnostic efficiency of REG Ia in chronic kidney disease (CKD). 38 39 Design 40 A cross-sectional study. 41 Setting Conducted in eastern China between August 2022 and August 2023. 42 **Participants** 43 A total of 880 participants aged over 18 years were enrolled, with 220 non-CKD 44 45 participants (111 in male, 50.45%) and 660 CKD patients (366 in male, 55.45%). CKD was diagnosed based on the Kidney Disease: Improving Global Outcomes (KDIGO) 46 2012 guidelines. Exclusion criteria included participation in other trials, acute kidney 47 injury, end-stage kidney disease undergoing renal replacement therapy, pregnancy, 48 49 active infections, gastrointestinal or pancreatic inflammation, history of gastrointestinal or pancreatic resections, cancer, and mental disorders. 50 51 Results Serum REG Ia was significantly higher in CKD group (125.54 [60.28-303.39] ng/mL) 52 53

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compared to those in non-CKD group (24.62 [14.09-37.32] ng/mL, P < 0.001). Positive correlations were observed between serum REG Ia and serum creatinine (Scr), cystatin 54 C (Cys-C), and kidney injury molecular-1(KIM-1), while a negative correlation was 55 identified with eGFR. After adjusting for sex, diabetes, hypertension, and fasting blood 56 57 glucose (FBG), the multivariate regression analysis demonstrated a significant association between serum REG Ia and eGFR (OR=1.737 [1.263-2.388], P = 0.001). 58 Furthermore, serum REG Ia levels increased progressively with declining kidney 59 function categorized by eGFR (P < 0.001). In CKD screening, serum REG Ia 60 demonstrated strong diagnostic performance, with an area under the receiver operating 61 62 characteristic curves (AUC) of 0.860 (0.813-0.899), providing a sensitivity of 71.63%,

2		
3 4	63	a specificity of 86.89%, a positive predictive value of (PPV) 94.30%, and a negative
5 6	64	predictive value (NPV) of 46.85%. Additionally, serum REG Ia exhibited an AUC of
7 8	65	0.769 (0.712-0.819) for identifying high and very-high risk CKD based on KDIGO risk
9 10	66	stratification. Its sensitivity significantly outperfomed serum Cys-C and KIM-1 (82.80%
11 12	67	vs 75.16% and 36.94%, respectively).
13 14	68	Conclusions
15 16	69	This study provided compelling evidence that serum REG I $\alpha$ levels were notably
17 18	70	elevated in patients with CKD and were closely associated with kidney function. REG
19 20	71	Ia may serve as as a promising biomarker for CKD detection and risk stratification.
21	72	Clinical trial registration
23	73	The study was approved by Ethics Committee of Zhongda Hospital (Approval Number:
24 25	74	2022ZDSYLL204-P01) and conducted in compliance with the Helsinki Declaration.
26 27	75	The clinical trial was registered under ChiCTR2300072247.
28 29	76	Data availability statement
30 31	77	The data underlying this article is available from the corresponding author under
32 33	78	reasonable request.
34 35	79	Patient and public involvement statement
36 37	80	It was not appropriate or possible to involve patients or the public in the design, or
38 39	81	conduct, or reporting, or dissemination plans of our research.
40 41	82	Strengths and limitations of this study
42 43	83	• This study utilized robust logistic regression models to adjust for confounding
44 45	84	factors and analyze the relationship between serum REG I $\alpha$ and kidney function.
46 47	85	• It was the first time to apply KDIGO risk stratification to explore the potential
48 49	86	association between serum REG Ia and the risk of CKD progression.
50 51	87	• The DeLong test was applied to statistically compare AUC values among the
52	88	biomarkers of CKD, enchancing the reliability of diagnostic performance
55 54	89	assessments.
56 57	90	Funding statement
58	91	This work was supported by the National Natural Science Foundation of China (No.
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- **Competing interests' statement** 
  - The authors declare that they have no competing interests.
- **Keywords** 
  - Regenerating protein Ia, Chronic kidney disease, Biomarker, Kidney function, Risk

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stratification;

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## 101 Abbreviations

List of abbreviations	
Area under the ROC	AUC
Blood urea nitrogen	BUN
Chronic kidney disease	CKD
Chronic kidney disease epidemiology collaboration	CKD-EPI
Cardiovascular disease	CVD
Cystatin C	Cys-C
Diabetic kidney disease	DKD
Estimate glomerular filtration rate	eGFR
End-stage kidney disease	ESKD
Fast blood glucose	FBG
Kidney disease improving global outcomes	KDIGO
Kidney injury molecular-1	KIM-1
Pancreatic stone protein	PSP
Regenerating protein Ia	REG Ia
Receiver operating characteristic curves	ROC
Serum creatinine	Scr
Standard deviation	SD
Uric acid	UA
Urine albuminuria creatine ratio	UACR
Unilateral ureteral obstruction	UUO

# 103 Introduction

Chronic kidney disease (CKD) encompasses a wide range of underlying etiologies and exhibits variable progression rates, <sup>1,2</sup> and may become the fifth leading cause of death worldwide by 2040.<sup>3</sup> The endpoint of CKD, known as end-stage kidney disease (ESKD), is characterized by a loss of approximately 90% of kidney function, rendering long-term survival without renal replacement therapy immpossible.<sup>2</sup> The high prevalence, low detection rate, severe clinical outcomes, and substantial econimic burden of CKD underscore its importance as a critical global health issue.<sup>4</sup> Early prevention, detection, and treatment are key to improving patient outcomes and slowing the progression to ESKD. 

Current biomarkers such as serum creatinine (Scr), blood urea nitrogen (BUN), estimate glomerular filtration rate (eGFR), and urine albuminuria creatine ratio (UACR) are routinely used to evaluate CKD severity.<sup>5-8</sup> In 2012, the Kidney Disease: Improving Global Outcomes (KDIGO) has taken the eGFR and UACR to create risk stratification strategies, assisting in the tailored management of CKD and guiding treatment for those at a higher risk of progression.<sup>9</sup> Emerging biomarkers, including cystatin C (Cys-C), kidney injury molecular-1 (KIM-1), and  $\beta$ 2-microglobuline, have demonstrated potential ability in enhancing the precision of CKD screening, either independently or in conjunction with traditional markers.<sup>2,3,10,11</sup> However, most biomarkers have not yet met clinical expectations in terms of sensitivity, specificity, and practicality.<sup>12-15</sup> Few biomarkers are capable of effectively detecting CKD while simultaneously assessing progression risk. Therefore, identifying a novel biomarker can both monitor kidney function decline and stratify CKD progression risk remains of paramount importance. 

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126 Regenerating protein I $\alpha$  (REG I $\alpha$ ), a 16 kDa protein primarily secreted by the pancreas 127 and intestine,<sup>16</sup> is also referred to as pancreatic stone protein (PSP).<sup>17</sup> It plays a vital 128 role in cellular proliferation and regeneration processes.<sup>18,19</sup> Recent studies have 129 reported the presence of REG I $\alpha$  in patients with various kidney diseases, suggesting 130 its involvement in renal pathology.<sup>20,21</sup> Our previous studies also have further 131 demonstrated that serum REG I $\alpha$  levels are elevated in patients with diabetic kidney

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disease (DKD), consistent with findings of H. Sobajima.<sup>22-24</sup> These observations
highlight the potential role of REG Iα as a biomarker for kidney insufficiency.

In this study, we aimed to investigate the relationship between serum REG Iα levels
and kidney function, assessed its potential as a screening tool for CKD, and evaluate its
role as a biomarker for kidney function and disease progression.

137 Methods

## 138 Study subjects

Participants were recruited from Zhongda Hospital between August 2022 and August
2023. The study was approved by the ethics committee (Approval Number:
2022ZDSYLL204-P01), with a clinical study registration of ChiCTR2300072247.
Informed consent was acquired from all participants.

The inclusion criteria were as follows: (1) non-CKD participant: age > 18 years; (2) CKD patients: age > 18 years and diagnosed with CKD in accordance to the Kidney Disease: Improving Global Outcomes (KIDGO) 2012 guidelines.<sup>1</sup> The exclusion criteria were as follows: (1) enrolled in another trial; (2) acute kidney injury; (3) patients with ESKD who are undergoing renal replacement therapy; (4) pregnancy; (5) active infection; (6) acute or chronic inflammation of the gastrointestinal system and pancreas; (7) history of gastrointestinal or pancreatic resections; (8) cancer; (9) mental disorders. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.<sup>25</sup> CKD stages were classified based on the U.S. National Kidney Foundation, while risk stratification of CKD progression was performed according to the KDIGO guideline.<sup>26,27</sup> Subgroup analyses were conducted among CKD patients to explore the relationship between serum REG Ia and different degrees of kidney function impairment.

156 A sample size calculation was conducted to ensure sufficient statistical power to detect 157 associations between serum REG I $\alpha$  and eGFR. The parameters included an expected 158 elevated REG I $\alpha$  proportion (P1=0.3), a two-tailed significance level ( $\alpha$ =0.05), 159 statistical power (80%), and finite population correction. A total of 880 participants 160 were included to enhance the robustness and generalizability of the study.

## 161 Data collection and quality assessment

Baseline demographics data were collected using standardized questionnaires. All participants have undergone 12-hours fasting and taken about 3mL of peripheral blood sample. Blood samples were centrifuged at 3500 rpm for 15 min, and the upper serum was collected within 6-hours. Serum samples were immediately stored at -80°C for subsequent analyses. Clinical biochemical parameters were extracted from the clinical laboratory of Hospital, such as Scr, BUN, uric acid (UA), Cys-C, Myoglobin, UACR, and fast blood glucose (FBG). The laboratory implements internal and external quality control procedures directed by a Chinese Quality Control Laboratory. The serum REG I $\alpha$  levels were determined using a double antibody sandwich ELISA, as previously described.<sup>28</sup> Serum KIM-1 was measured using an ELISA kit (KE00136) from Proteintech.

### 173 Statistical analysis

Statistical analyses were conducted using SPSS 20.0, Med-Calc, and GraphPad Prism 8.0. Continuous variables were summarized as mean  $\pm$  standard deviation (SD) for normally distributed data or as median with interguartile range (IOR) for non-normally distributed data. For categorical variables, the frequency with a percentage of each category was calculated. Normality was assessed using graphical methods (Q-Q plots) and the Shapiro-Wilk test. For variables that did not meet normality, we used non-parametric descriptors and methods. We used Student's t test for normally distributed continuous variables, Mann Whitney U test for non-normally distributed continuous variables, and chi-square or Fisher's exact test for categorical variables for two group comparisons. Tukey's multiple comparison test was employed to examine the differences in biomarker values across the three or more groups, thereby avoiding the issue of multiple comparisons. Spearman's rank correlation analyses and ordinal logistic regression was used to measure the associations between serum REG Ia and other biomarkers of kidney function. Multivariate logistic regression analysis was utilized to identify the independent factors of kidney dysfunction. The study included age, BUN, UA, serum myoglobin, serum Cys-C, serum KIM-1/100, serum REG Iα/100 Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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into ordinal multiple logistic regression model, while adjusting for sex, diabetes, hypertension, and FBG. The multivariate logistic regression model also incorporates the above covariates. The area under the receiver operating characteristic curve (AUC) was plotted to analyze the ability of serum Cys-C, KIM-1, and REG Ia to screen the patients with CKD, and detect the high and very-high risk patients. The study assessed evaluate accuracy using sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy, and applied the DeLong test to statistically analyze AUC differences between receiver operating characteristic curves. *P* value of <0.050 using two-tailed tests was considered statistically significant.

**Results** 

#### 200 Baseline characteristics of the study population

Overall, a total of 880 participants were enrolled, comprising 220 non-CKD participants and 660 CKD patients (Supplementary data Figure 1). Significant differences were observed between CKD patients and non-CKD participants in terms of age, complication diseases (diabetes and hypertension), and kidney function biomarkers (Scr, BUN, UA, eGFR, Cys-C, and KIM-1), and serum myoglobin (all P < 0.001, Table 1). Serum REG Iα levels were significantly elevated in CKD patients (125.54 [60.28, 303.39] ng/mL) compared to non-CKD participants (24.62[14.09, 37.32] ng/mL, P <0.001, Table 1). Biomarker trends revealed that serum Cys-C, REG Ia, and myoglobin levels progressively increased as eGFR declined (Figure 1: A1, C1, and D1). Serum KIM-1 levels also exhibited significant differences between non-CKD group and CKD groups (G2 to G5, P < 0.010, Figure 1: B1). Further analyses confirmed that cardiovascular disease (CVD, Supplementary data Figure 2) did not influence the distribution of serum REG Ia and myoglobin levels. Diabetes had no influence on serum REG Ia in CKD patients (Supplementary data Figure 3). 

	Non-CKD Participants	<b>CKD</b> Patients	<i>P</i> Valu
Number	220	660	-
DEMOGRAPHICS			
Age (years)	53 (40-62)	62 (50-72)	< 0.00
Sex (male, %)	111 (50.45)	366 (55.45)	0.212
Smoking (yes, %)	41 (18.64)	142 (21.52)	0.14
Drinking (yes, %)	30 (13.64)	84 (12.73)	0.823
COMPLICATION DISEASES			
Diabetes (yes, %)	75 (34.09)	350 (53.03)	< 0.00
Hypertension (yes, %)	65 (29.55)	501 (75.91)	< 0.00
Cardiovascular disease (CVD, yes, %)	44 (20.00)	149 (22.58)	0.45
LABORATORY MEASUREMENTS			
Fast blood glucose (FBG, mmol/L)	5.45 (4.99-6.67)	5.34 (4.57-7.11)	0.00
Serum creatinine (Scr, µmol/L)	64.00 (55.25-76.00)	126.00 (83.00-418.50)	< 0.00
Blood urea nitrogen (BUN, mmol/L)	5.20 (4.30-6.28)	10.30 (7.00-18.60)	< 0.00
Serum uric acid (UA, µmol/L)	301.00 (256.00-365.25)	354.00 (290.00-443.00)	< 0.00
Estimated glomerular filtration rate (eGFR, mL/min per 1.73 m <sup>2</sup> )	102.37 (95.73-112.36)	44.03 (10.96-77.09)	< 0.00
Serum cystatin C (Cys-C, mg/L)	1.10 (0.99-1.24)	1.79 (1.23-3.50)	< 0.0
Serum kidney injury molecular-1 (KIM-1, pg/mL)	186.28 (57.22-266.88)	247.72 (175.10-334.13)	< 0.00
Serum Myoglobin (ng/mL)	46.81 (33.49-58.00)	64.04 (35.96-112.51)	< 0.0
Serum regenerating protein Iα (REG Iα, ng/mL)	24.62 (14.09-37.32)	125.54 (60.28-303.39)	< 0.0

зy i y y continuous variables. And chi-square or Fisher's exact tests were used for categorical variables for two group comparisons.

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220	<b>Relationship between s</b>	serum REG Ia and	kidney function
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221 This study explored the relationship between serum REG Ia levels and kidney function 222 biomarkers. In figure 2, Spearman correlation analyses demonstrated a strong positive 223 association between serum REG Ia and Scr (r = 0.753, P < 0.001), BUN (r = 0.733, P< 0.001), serum Cys-C (r = 0.678, P < 0.001), and serum KIM-1 (r = 0.217, P < 0.001). 224 And a siginificant negative correlation was observed between serum REG Ia and eGFR 225 (r = -0.789, P < 0.001). A comprehensive summary of these correlations was provided 226 227 in supplementary data Table 1. Ordinal logistic regression analysis carried out in all participants, with eGFR as a grade-dependent variable, revealed that serum REG I $\alpha$ /100 228 -a .ted with levels was significantly associated with eGFR (OR=1.737, 95% CI: 1.263-2.388, P =229 230 0.001, Table 2).

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231	Table 2. The logistic regression analyses showing the relationship between variables
232	and kidney function

	Ordinal Logistic Regression <sup>#</sup>		Multivariate Logistic Regression	
	<i>P</i> Value	OR (95% CI)	P Value	OR (95% CI)
Age <sup>a</sup>	0.050	1.020 (1.001-1.041)	0.009	0.966 (0.942-0.992)
BUN <sup>b</sup>	< 0.001	1.266 (1.165-1.376)	< 0.001	1.440 (1.216-1.706)
UA°	0.337	1.001 (0.999-1.004)	0.085	1.003 (1.000-1.007)
Serum Myoglobin <sup>d</sup>	0.165	1.005 (0.998-1.013)	0.148	1.136 (0.897-1.559)
Serum Cys-C <sup>e</sup>	< 0.001	6.784 (4.016-11.460)	0.071	1.853 (0.949-3.620)
Serum KIM-1/100 <sup>f</sup>	0.133	1.069 (0.980-1.167)	0.122	1.243 (0.943-1.639)
Serum REG Ia/100 <sup>d</sup>	0.001	1.737 (1.263-2.388)	0.022	1.799 (1.088-2.975)

233 #: The ordinal multiple logistic regression showing variables independently associated with eGFR levels 234 in all participants. \*: The multivariate logistic regression analysis identified the independent influencing 235 factors for high and very-high risk patients with CKD in accordance with KDIGO risk stratification. The 236 analyses included age, BUN, UA, serum myoglobin, serum Cys-C, serum KIM-1/100, serum REG 237 Iα/100 into ordinal multiple logistic regression model, while adjusting for sex, diabetes, hypertension, 238 and FBG. The multivariate logistic regression model also incorporates the above covariates.

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239 FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1:

240 kidney injury molecular-1, REG Ia: regenerating protein Ia, eGFR: estimated glomerular filtration rate,

241 CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

242 a: years, b: mmol/L, c: µmol /L, d: ng/mL, e: mg/L, f: pg/mL.

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# 243 Subgroup analysis in patients with CKD

To explore the relationship between serum REG Ia levels and CKD progression risk, 256 CKD patients were included in the sub-research and classified by eGFR and UACR levels in accordance with KDIGO risk stratification guideline.<sup>1</sup> The patients were stratified into four KDIGO risk groups: low risk (18.00%), moderate risk (20.70%), high risk (17.20%), and very-high risk (44.10%, supplementary data Table 2). Serum REG Ia levels significantly increased with higher CKD risk categories, reaching 184.38 (108.81, 314.71) ng/mL in the very-high risk group (P < 0.001, Figure 1: C2). Similar trends were observed for serum Cys-C (2.57 [1.94, 3.53] mg/L) and myoglobin (67.00 [51.70, 113.68]ng/mL, P < 0.001, Figure 1: A2, D2). However, the serum KIM-1 did not exhibit an increasing trend (P > 0.050, Figure 1: B2). Multiple logistic regression analysis demonstrated that serum REG  $I\alpha/100$  was an independent influencing factor for high and very-high CKD risk (OR=1.799, 95% CI: 1.088-2.975, P = 0.022, Table 2). 

# 257 Ability of serum REG Iα in screening patients with kidney dysfunction

Receiver operating characteristic (ROC) analysis evaluated the utility of serum REG Iα as a screening tool for CKD and its ability to stratify CKD risk (Figure 3 and supplementary data Table 3). Serum REG Ia demonstrated an AUC of 0.860 (95% CI: 0.813-0.899) for detecting CKD, comparable to serum creatinine (0.850, 95% CI: 0.801-0.890) and serum Cys-C (0.842, 95% CI: 0.793-0.883). At a cutoff value of 70.82 ng/mL, serum REG Ia had a sensitivity of 71.63%, specificity of 86.89%, positive predictive value (PPV) of 94.30%, and negative predictive value (NPV) of 46.85%. Serum KIM-1 showed a lower AUC than serum REG Iα, measuring 0.714 (95% CI: 0.656-0.767, P < 0.001). Serum myoglobin had the lowest AUC among the five biomarkers, measuring 0.642 (95% CI: 0.581-0.699, *P* < 0.001). 

For distinguishing high and very-high CKD risk, serum REG Iα demonstrated
superior performan compared to serum KIM-1 (AUC = 0.769 [0.712-0.819] vs 0.528
[0.465-0.590], P < 0.010, supplementary data Table 3). Serum Cys-C had the highest</li>
AUC (0.865 [0.817-0.904], P < 0.010) among the three biomarkers. Serum REG Iα,</li>

with a cutoff value of 76.05 ng/mL, exhibited sensitivity of 82.80%, specificity of
62.63%, PPV of 77.38%, and NPV of 69.32%. Notably, serum REG Iα showed
significantly higher sensitivity than serum Cys-C and KIM-1.

275 Discussion

This study was the first time to systematically evaluate serum REG I $\alpha$  levels in a broad CKD population and confirm its upregulation in CKD patients. First, serum REG Ia levels increased progressively with declining eGFR and correlated strongly with conventional kidney function biomarkers (Scr, BUN, Cys-C, and KIM-1). Second, serum REG Ia emerged as an independent risk factor for patients classified as high and very-high risk according to the KDIGO risk stratification. Third, serum REG Ia demonstrated robust diagnostic performance, showing higher sensitivity than serum KIM-1 for identifying CKD and distinguishing risk progression. 

REG Ia, a low molecular weight protein (16 kDa), was initially discovered in pancreas, and was identified as pancreatic stone protein (PSP) due to its role in inhibiting calcium carbonate stone formation in pancreatic ducts.<sup>29,30</sup> Immunohistochemical studies have shown overexpression of REG Ia in impaired kidneys, particularly in proximal tubules and thick ascending limbs of Henle's loops.<sup>20</sup> Previous studies also reported elevated REG Ia levels in diabetic kidney disease (DKD), suggesting its involvement in tubular dysfunction and kidney injury.<sup>22,31,32</sup> Our previous studies provided evidence, that serum REG Ia levels were notably elevated in patients with type 2 diabetes mellitus who had kidney dysfunction. And we also observed that pregnant women with early renal dysfunction exhibited similar elevations in serum REG Ia levels.<sup>24,33</sup> Notably, the serum REG Ia levels remained significantly elevated when the scope of this study was expanded to the entire CKD population. 

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We incorporated myoglobin (17.6 kDa)<sup>34</sup> to investigate whether the elevated level of
serum REG Iα represents a universal phenomenon of accumulation in CKD. The
myoglobin was identified as a biomarker for acute myocardial ischemia and
rhabdomyolysis.<sup>35</sup> Some studies indicated that serum myoglobin levels increase in

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kidney impairment. This accumulation was primarily attributed to slower clearance in CKD.<sup>36</sup> We showed that serum levels of myoglobin have no differences between non-CKD participants and patients with early stage of CKD (group G1 and group G2). And there was a gradual increase in patients with medium to end-stages of CKD (group G3 to group G5). The possible reason was as follows: in the early stages of kidney injury, compensatory glomerular hypertrophy with hyperfiltration state was known to exist. This mechanism allowed some low molecular weight protein to be filtered through the glomerular basement membrane, resulting in no discernible difference during the early stage of CKD (eGFR  $\ge$  60 mL/min per 1.73 m<sup>2</sup>), and even appearing as a transient decrease. With progression to medium to end-stages of CKD  $(eGFR < 60 \text{ mL/min per } 1.73 \text{ m}^2)$ , the glomerular basement membrane thickened and led to significant decline in glomerular filtration function, ultimately causing proteins accumulation in serum.<sup>37</sup> The serum levels of REG Iα showed a gradual increase in medium to end-stages of CKD, which was similar to the rise observed in serum myoglobin levels. However, it exhibited a significant increase in the early stage of CKD, which was different with serum myoglobin. This suggested that, in addition to the sensitive accumulation of serum REG Ia as a low molecular weight protein, there might be a specific production when it comes to kidney impairment. The unique behavior of serum REG I $\alpha$ , compared to low molecular weight protein, highlights its dual role: accumulation due to reduced glomerular clearance and potential increased production in response to kidney injury. Unlike myoglobin, which remains stable in early CKD stages, serum REG Ia levels were significantly elevated even at the ultra-early stage of kidney dysfunction, suggesting a sensitive and specific response to renal impairment.

325 The etiology underlying the upregulation of REG I $\alpha$  production in CKD patients 326 remains elusive. Two potential mechanisms are considered as follows. First, REG I $\alpha$ 327 resists apoptosis and promotes cell proliferation in different inflammation 328 situations.<sup>38-42</sup> Studies have shown that cytokines such as IL-6 can increase the 329 proliferation of REG I $\alpha$ , which is involved in cell regeneration and repair.<sup>42,43</sup> In

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CKD, the occurrence and progression of the disease are closely related to the expression of multiple inflammatory cytokines, including IL-1, IL-6, and TNF-a. <sup>37,44,45</sup> Under the stimulation of chronic inflammation, different types of renal cells secrete REG Ia locally to participate in kidney anti-apoptosis and proliferation and against kidney fibrosis in the development of CKD. The secreted REG Ia enters the circulation through reabsorption by kidney tubules, resulting in a significant increase in serum REG Ia levels. Thus, REG Ia might serve as an inflammatory factor involved in kidney diseases. Second, REG Ia is primarily synthesized in the pancreas and released into the circulation.<sup>18,46</sup> A hypothesis suggests that a cross-talk mechanism exists between the pancreas and kidney when renal damage occurs. This interaction may prompt the pancreas to produce more REG I $\alpha$  in response to kidney injury, resulting the elevation in serum. Although the exact cause of REG I $\alpha$ upregulation remains unclear, these two proposed mechanisms provided potential insights into the relationship between REG Ia and CKD. 

At present, the assessment of CKD generally focused on glomerular filtration capacity, which is characterized by Scr. UACR, and eGFR.<sup>2</sup> The stabilities of these factors are compromised by age, dietary intake, and physical activity. In general, the performance of eGFR is poor for populations outside of North America, Australia, and Europe, and accuracy decreases at the extremes in the distribution of age, and body composition.<sup>7</sup> Therefore, new biomarkers of kidney impairment have been discovered. Cys-C is produced by all nucleated cells at a steady rate and freely filtered by the kidney.<sup>47</sup> The accumulation of its levels is observed in case of glomerular filtration dysfunction, with limited impact and strong stability.<sup>48</sup> Eventually, serum Cys-C is incorporated into the eGFR equation as a reliable and sensitive indicator of kidney function. The sensitivity of serum Cys-C is heightened in the early stages of CKD compared to creatinine.<sup>49,50</sup> Another biomarker is KIM-1,<sup>51,52</sup> which is secreted following kidney proximal tubular injury, and has emerged as a sensitive and specific biomarker of acute kidney injury and CKD progression.<sup>53-</sup> <sup>56</sup> Compared to serum KIM-1, the REG I $\alpha$  had several advantages as follows. This 

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paper demonstrated that levels of serum REG Ia increase significantly earlier than KIM-1, making it a better marker for the early detection of renal injury. In addition, serum REG Ia was sensitive in distinguishing between different stages of CKD. Its ability to discriminate early from advanced stages of CKD provides valuable diagnostic and prognostic information. Serum REG Ia also exhibited better AUC, sensitivity, and specificity, enhancing its diagnostic performance in identifying CKD patients. In summary, the advantages of serum REG Ia over serum KIM-1 primarily lie in its ability to detect renal injury earlier, its sensitivity in differentiating various stages of CKD, and its better diagnostic performance in identifying CKD patients. These features collectively underscore its potential as a potential biomarker for CKD. In this study, serum REG Ia strongly correlated with serum creatinine and Cys-C, and had a similar performance to serum creatinine and Cys-C in detecting patients with CKD. The serum levels of REG Ia were more likely related to glomerular filtration capacity according to the correlation analyses, although the mechanism of its production and degradation or excretion in CKD remained unclear. We indicated that serum REG Ia might be more sensitive than serum creatinine and Cys-C in detecting ultra-early stage of kidney dysfunction. Similar phenomenon was observed in distribution analyses in KDIGO risk stratification. The KDIGO risk classification was widely used in evaluating the progression and prognosis risk in patients with CKD.<sup>57-60</sup> Serum levels of REG Ia gradually elevated with higher KDIGO risk stratification categories. And it emerged as an independent risk factor for patients with CKD categorized as high and very-high risk. Our findings provided a new insight that serum REG Ia performed better than serum KIM-1 in screening patients with CKD and detecting high and very-high risk patients with CKD. Moreover, serum REG Ia displayed a highest sensitivity in identifying high and very-high risk CKD. These results highlight the potential application of serum REG Ia as a valuable biomarker in the screening of patients with CKD and the assessment of CKD risk. We observed that patients with CKD were older than non-CKD participants, and they had higher rates of diabetes and hypertension. This finding was consistent with the 

typical etiology of CKD. Globally, diabetes and hypertension are recognized as the primary causes of CKD.<sup>2</sup> Diabetes accounts for 30-50% of all CKD cases and affects approximately 285 million adults worldwide. A consistent finding in observational studies shows that the increased risk of developing is associated with blood pressure control. These findings were consistent with the expected proportion of CKD etiology in our study.

There are some limitations in this study. First, the cross-sectional design precluded causal inference. And due to the challenges of obtaining detailed data of renal biopsy, our team have endeavored to conduct a pre-subgroup analysis within the available constraints. We found that CKD etiologies (for example, IgA nephropathy, membranous nephropathy, and diabetic kidney disease) have no effect on serum REG levels. To enhance causality and generalizability, longitudinal designs with larger sample sizes and diverse populations should be considered in forthcoming research. Second, although robust biomarkers such as creatinine and Cys-C are already available for the diagnosis of CKD, the potential efficacy of REG Ia as a combined biomarker is anticipated to be revealed in subsequent large-scale analyses. Third, the precise source of elevated REG I $\alpha$  in CKD remains unclear, warranting further mechanistic investigations. Forth, to address residual confounding, future studies should incorporate additional covariates such as drug use and different causes of CKD and conduct sensitivity analyses to assess potential biases. 

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Moreover, we acknowledged the potential impact of self-reporting and recall bias in our questionnaire-based demographic data, especially among CKD patients prone to cognitive and emotional challenges. Factors such as cognitive impairment, symptom complexity, emotional stress, health literacy variations, and medication effects can skew reporting accuracy. To mitigate risks, we used standardized questionnaires with clear instructions. However, given CKD's clinical complexity, findings should be interpreted cautiously. Future studies should cross-verify self-reported data with objective clinical measures to enhance accuracy and reliability. 

<sup>59</sup> 60 416 **Conclusion** 

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417 This study provided compelling evidence that serum REG Iα is significantly
418 upregulated in CKD patients and strongly associated with kidney function. Serum
419 REG Iα demonstrated notable diagnostic sensitivity and utility in CKD risk
420 stratification, underscoring its potential as a valuable biomarker for detecting kidney
421 function decline and identifying high risk CKD patients.

422 Contributorship statement

Professor Ling Li acted as guarantor. Ling Li conceptualized the study; Bin Wang reviewed and edited the manuscript; Nan Huang and Xiangyun Zhu were responsible for the data analysis and wrote the original draft. Nan Huang, Sheng Chen, Xiaodong Wu, Hui Wang, Zhiyi Shu and Xi Huang were responsible for inclusion of population, collection of samples and data, and the experiments. Jianling Bai and Jinfang Sun were responsible for guidance on data analysis. Pingsheng Chen and Xiuxiu Hu were responsible for analysis of kidney biopsy tissues from CKD patients. Rolf Graf provided the excellent technical support for this study. 

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# 611 **Tables**

Table 1. Clinical characteristics of study population at baseline examination.

613 CKD: chronic kidney disease; The data were presented in quartiles. Student's t tests were used for
614 normally distributed continuous variables. Mann Whitney U tests were used for non-normally
615 distributed continuous variables. And chi-square or Fisher's exact tests were used for categorical
616 variables for two group comparisons.

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1 2 2

618 Table 2. The logistic regression analyses showing the relationship between variables619 and kidney function.

#: The ordinal multiple logistic regression showing variables independently associated with eGFR
levels in all participants. \*: The multivariate logistic regression analysis identified the independent
influencing factors for high and very-high risk patients with CKD in accordance with KDIGO risk
stratification. The analyses included age, BUN, UA, serum myoglobin, serum Cys-C, serum KIM1/100, serum REG Iα/100 into ordinal multiple logistic regression model, while adjusting for sex,
diabetes, hypertension, and FBG. The multivariate logistic regression model also incorporates the
above covariates.

627 FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM628 1: kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration

629 rate, CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

630 a: years, b: mmol/L, c:  $\mu$ mol /L, d: ng/mL, e: mg/L, f: pg/mL.

632 Figures

631

633 Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum634 Myoglobin in different groups.

A1, B1, C1, and D1 are in all participants: the CKD groups were classified in accordance with
eGFR levels as described in methods. A1: Distribution of serum Cys-C. B1: Distribution of
serum KIM-1. C1: Distribution of serum REG Iα. D1: Distribution of serum myoglobin. A2, B2,
C2, and D2 are in patients with CKD: the patients were classified in accordance with 2012
KDIGO risk stratification as described in methods. A2: Distribution of serum Cys-C. B2:

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640 Distribution of serum KIM-1. C2: Distribution of serum REG Iα. D2: Distribution of serum
641 Myoglobin. \*: P < 0.050, \*\*: P < 0.010, \*\*\*: P < 0.001. Tukey's multiple comparison tests were</li>
642 conducted to examine the differences in different groups. CKD: chronic kidney disease, KDIGO:
643 kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1,
644 REG Iα: regenerating protein Iα.

645

646 Figure 2. Correlations between serum REG I $\alpha$  and different markers in all 647 participants.

648A: correlation between serum REG Iα and Scr (r = 0.753, P < 0.001), B: correlation between649serum REG Iα and BUN (r = 0.733, P < 0.001), C: correlation between serum REG Iα and serum650Cys-C (r = 0.678, P < 0.001), D: correlation between serum REG Iα and serum KIM-1 (r = 0.217,651P < 0.001), E: correlation between serum REG Iα and eGFR (r = -0.789, P < 0.001). Scr: serum652creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1,653eGFR: estimated glomerular filtration rate, REG Iα: regenerating protein Iα.

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655 Figure 3. Ability of screening patients with CKD.

656 The AUC of serum REG Ia was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 657 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 658 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% CI: 659 0.581-0.699). The AUC of serum REG I $\alpha$  was similar to serum Creatinine and serum Cys-C (P > 660 0.050), and was significantly higher than serum KIM-1 and serum Myoglobin (P < 0.001). DeLong 661 tests were applied to analyze AUC differences between receiver operating characteristic curves. CKD: 662 chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Ia: 663 regenerating protein Ia, Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

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665 Supplementary material

666 Supplementary data Table 1. Correlations between serum REG Iα and different667 markers in all participants.

3 4	668	**: $P < 0.050$ , ***: $P < 0.001$ . CVD: cardiovascular disease, FBG: fast blood glucose, Scr: serum
5 6 7	669	creatinine, BUN: blood urea nitrogen, UA: serum uric acid, eGFR: estimated glomerular filtration
8 9	670	rate, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα.
10 11	671	
12 13	672	Supplementary data Table 2. Distribution of serum Cys-C, serum KIM-1, serum REG
14 15	673	I $\alpha$ and serum Myoglobin in KDIGO risk stratification.
16 17	674	Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ι α: regenerating protein Ι α, KDIGO:
18 19	675	kidney disease improving global outcomes. The data were presented in quartiles.
20 21	676	
22 23	677	Supplementary data Table 3. Ability of different biomarkers to screen patients with
24 25	678	CKD.
26 27	679	***: Comparing with serum REG Ia, $P < 0.001$ . DeLong tests were applied to analyze AUC
28 29 20	680	differences between receiver operating characteristic curves. CKD: chronic kidney disease, KDIGO:
30 31 22	681	kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1,
32 33 34	682	REG I $\alpha$ : regenerating protein I $\alpha$ . AUC: area under the receiver operating characteristic curve,
35 36	683	PPV: positive predictive value, NPV: negative predictive value.
37 38	684	
39 40	685	Supplementary data Figure 1. Flowchart of participant selection.
41 42	686	In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the
43 44	687	exclusion criterion, participants were excluded because of missing critical data (n=108), active
45 46	688	infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy
47 48	689	(n=7), and mental disorders (n=6). CKD: chronic kidney disease.
49 50	690	
50 51 52	691	Supplementary data Figure 2. Distribution of serum REG I $\alpha$ and Myoglobin in non-
52 53 54	692	CKD and different CKD groups, which participants without CVD.
55 56	693	A: Distribution of serum REG Ia. The serum REG Ia level of non-CKD group was significantly lower
57 58	694	than that of G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin. The serum
59 60	695	myoglobin level of non-CKD was significantly lower than that of G4 and G5 groups individually.
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4 5	696	CKD: chronic kidney disease, CVD: cardiovascular disease, REG I $\alpha$ : regenerating protein I $\alpha$ . *: $P <$
6 7 8	697	0.050, **: $P < 0.010$ , ***: $P < 0.001$ . Tukey's multiple comparison tests were conducted to examine
9 10	698	the differences in different groups.
11 12	699	
13 14	700	Supplementary data Figure 3. Levels of serum REG I $\alpha$ in CKD patients with and
15 16	701	without diabetes.
10 17 18	702	Mann-Whitney U test was used for non-normally distributed continuous variables ( $P = 0.121$ ). REG
19         20         21         22         23         24         25         26         27         28         29         30         31         32         33         34         35         36         37         38         39         40         41         42         43         44         45         46         47         48         49         50         51         52         53         54         55         56         57         58         59	703	Ια regenerating protein Ια
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Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum Myoglobin in different groups.

A1, B1, C1, and D1 are in all participants: the CKD groups were classified in accordance with eGFR levels as described in methods. A1: Distribution of serum Cys-C. B1: Distribution of serum KIM-1. C1: Distribution of serum REG Ia. D1: Distribution of serum myoglobin. A2, B2, C2, and D2 are in patients with CKD: the patients were classified in accordance with 2012 KDIGO risk stratification as described in methods. A2: Distribution of serum Cys-C. B2: Distribution of serum KIM-1. C2: Distribution of serum REG Ia. D2: Distribution of serum Myoglobin. \*: P < 0.050, \*\*: P < 0.010, \*\*\*: P < 0.001. Tukey's multiple comparison tests were conducted to examine the differences in different groups. CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ia: regenerating protein Ia.

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Figure 2. Correlations between serum REG Ia and different markers in all participants.

A: correlation between serum REG Ia and Scr (r = 0.753, P < 0.001), B: correlation between serum REG Ia and BUN (r = 0.733, P < 0.001), C: correlation between serum REG Ia and serum Cys-C (r = 0.678, P < 0.001), D: correlation between serum REG Ia and serum KIM-1 (r = 0.217, P < 0.001), E: correlation between serum REG Ia and eGFR (r = -0.789, P < 0.001). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG Ia: regenerating protein Ia.







Figure 3. Ability of screening patients with CKD.

The AUC of serum REG I $\alpha$  was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% CI: 0.581-0.699). The AUC of serum REG I $\alpha$  was similar to serum Creatinine and serum Cys-C (P > 0.050), and was significantly higher than serum KIM-1 and serum Myoglobin (P < 0.001). DeLong tests were applied to analyze AUC differences between receiver operating characteristic curves. CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG I $\alpha$ : regenerating protein I $\alpha$ , Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

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Spearman Analyses (r)	able 1. C	2 2	ons betwo	een serur	n REG lo	$\alpha$ and diff	rerent ma	8 8	all partic	10 ant Seign sreign	bruary 11	12	13	
Age (1)	1.000		$\sim$							ement ted to	025. D			
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Hypertension (4)	0.262***	0.087**	0.236***	1.000						a mini				
CVD (5)	0.302***	0.024	0.125***	0.234***	1.000					ng, Al	:tp://br			
FBG (6)	0.258***	0.003	0.567***	0.050	0.062	1.000				trainir	njoper			
Scr (7)	0.037	0.294***	0.030	0.409***	0.098***	-0.159***	1.000			ıg, and	1.bmj.			
BUN (8)	0.157***	0.170***	0.136***	0.442***	0.125***	-0.072**	0.845***	1.000		d simil	com/ o			
UA (9)	-0.086**	0.267***	0.008	0.137***	0.036	-0.051	0.420***	0.379***	1.000	ar tec	n Jun			
eGFR (10)	-0.245***	-0.085**	-0.081**	-0.464***	-0.148***	0.123***	-0.927***	-0.856***	-0.328***	1.00 <b>00</b>	e 14, 2			
Serum Cys-C (11)	-0.073	0.048	-0.237***	0.034***	0.079	-0.251***	0.811***	0.741***	0.336***	-0.82 <b>6</b> **	025 1.000			
Serum KIM-1 (12)	0.069	0.034	0.141***	0.078	0.031	0.105**	0.091**	0.121***	-0.076	-0.111**	t Agen 0.111**	1.000		
Serum Myoglobin (13)	0.138***	0.117***	0.098**	0.229***	0.153***	0.024	0.648***	0.606***	0.210***	-0.661***	<b>Ce</b> 0.492***	0.066	1.000	

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3 of 46			BMJ Open	jopen-2024 by copyrig			
	<b>Serum REG Ια (14)</b> 0.154*** 0	0.059 0.073** 0.369*** 0.0	099*** -0.134*** 0.753*** 0.733	•••• 0.275*** -0.78 <sup>6</sup> ••• 130.67	8*** 0.217*** 0.565*** 1.0		
3	**: P < 0.050, ***: P < 0.001. CVD: car	diovascular disease, FBG: fast t	plood glucose, Scr: serum creatini	<u>ع</u> بة الله ne, BUN: blood urea المناقبة الم	UA: serum uric acid, eGFR: e		
4 5	glomerular filtration rate, Cys-C: cystati	n C, KIM-1: kidney injury mole	ecular-1, REG Iα: regenerating pro	ry 2025. Prelated to te to te			
6	Supplementary data Table 2. Dist	ribution of serum Cys-C, s	and serum Myseland serum Myseland serum Myseland serum Myseland serum Myseland serum Myseland serum market serum ser	☆등 클 활동 obin in KDIGO risk stratificat			
	KDIGO	Low Risk	Moderate Risk		Very-high Risk		
	<b>Risk Stratification Groups</b>	(18.00%)	(20.70%)		(44.10%)		
	Serum Cys-C (mg/L)	1.23 (1.01-1.43)	1.22 (1.05-1.45)	<b>9</b> • <b>9</b> 1.54 (1.17-1≱9)	2.57 (1.94-3.53)		
	Serum KIM-1 (pg/mL)	255.77 (211.33-279.86)	262.46 (205.21-312.24)		247.28 (142.72-417.99)		
	Serum Myoglobin (ng/mL)	29.30 (18.97-46.25)	41.50 (22.39-51.82)	30.25 (20.60- <b>2</b> ).52	67.00 (51.70-113.68)		
	Serum REG I a (ng/mL)	48.86 (34.18-78.28)	76.05 (51.09-120.54)	79.18 (58.64-1	184.38 (108.81-314.71)		
7	Cys-C: cystatin C, KIM-1: kidney injury	$\gamma$ molecular-1, REG I $\alpha$ : regene	erating protein Ι α, KDIGO: kidn	ey disease improving logal ou	itcomes.		
8 9	The data were presented in quartiles.			⊧ 14, 2025 at Agence Bibli nologies.			
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0	Supplementary data Table 3. Al	oility of differe	nt biomarkers to scre	een patients v	vith CKD.		4 on 13 luding fo		
	Varial	bles	AUC (95% CI)	Cutoff	Sensitivity	Specificity	r e <b>P</b> PV Ense	NPV	
				value	(%)	(%)	elate	(%)	
			Ability of screening pa	atients with Ck	XD in all partic	cipants	5. Do nent d to t		
	Serum REG I	α (ng/mL)	0.860 (0.813-0.899)	70.82	71.63	86.89	ext al	46.85	
	Serum Creatinii	ne (µmol/L)	0.850 (0.801-0.890)	88.00	69.23	96.72	nd dat	47.90	
	Serum Cys-C	C (mg/L)	0.842 (0.793-0.883)	1.33	72.12	91.80	a min.57	49.09	
	Serum KIM-1	(pg/mL) (	).714 (0.656-0.767) ***	232.98	63.94	68.85	ing, A	35.09	
	Serum Myoglol	oin (ng/mL)	0.642 (0.581-0.699) ***	59.70	48.08	81.97	trainii	30.72	
	Ability of	screening high ar	nd very-high risk patier	nts according to	o KDIGO risk	stratification	ing CED partie	cipants	
	Serum REG I	a (ng/mL)	0.769 (0.712-0.819)	76.05	82.80	62.63	d simi	69.32	
	Serum Cys-C	C (mg/L) (	).865 (0.817-0.904) ***	1.63	75.16	92.93	on 950.65	70.00	
	Serum KIM-1	(pg/mL) (	).528 (0.465-0.590) ***	327.10	36.94	87.88	e 14.69	46.49	
1	***: Comparing with serum REG Iα, I	P < 0.001. DeLong	g tests were applied to an	alyze AUC diff	erences betwee	en receiver ope	rating character	ristic curves. CKD	): chronic kidney
2	disease, KDIGO: kidney disease impr	oving global outco	omes, Cys-C: cystatin C,	, KIM-1: kidney	injury molecu	lar-1, REG I	α: regenerating	protein Ι α. AU	C: area under the
3	receiver operating characteristic curve	, PPV: positive pr	edictive value, NPV: neg	gative predictiv	e value.		ce Bib		
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Supplementary data Figure 1. Flowchart of participant selection.

In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental disorders (n=6). CKD: chronic kidney disease.



Supplementary data Figure 2. Distribution of serum REG Iα and Myoglobin in non-CKD and different CKD groups, which participants without CVD.

A: Distribution of serum REG Ia. The serum REG Ia level of non-CKD group was significantly lower than that of G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower than that of G4 and G5 groups individually. \*: P < 0.050, \*\*: P < 0.010, \*\*\*: P < 0.001. Tukey's multiple comparison tests were conducted to examine the differences in different groups. CKD: chronic kidney disease, CVD: cardiovascular

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Supplementary data Figure 3. Levels of serum REG I $\alpha$  in CKD patients with and without diabetes. Mann-Whitney U test was used for non-normally distributed continuous variables (*P* = 0.121). REG

Iα: regenerating protein Iα.