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## Cardiovascular disease risk of repetitive proteinuria in multiple-time kidney screenings among middle-to-older age general population: a cohort study

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1	Cardiovascular disease risk of repetitive proteinuria in multiple-time kidney screenings
2	among middle-to-older age general population: a cohort study
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23 ABSTRACT

Objectives: We aimed to investigate the association between repetitive proteinuria and
 cardiovascular events among middle-to-older general Japanese population.

**Design:** Cohort design

Setting: We used repeated health screening results and medical claim data from one of thelargest health insurers in Japan.

**Participants:** Among the middle-aged and older participants (40-74 years old, n=179,840), 90,752 were excluded for undertaking health screening fewer than two times and 344 were excluded for having a history of cardiovascular diseases; 88,744 who underwent kidney function screenings at least twice (from April 2011 to March 2015) were included in the analysis. Based on dipstick proteinuria test results, they were divided into repetitively positive (positive [positive proteinuria was defined as  $\geq$ 1+] twice or more), once-positive, and all-negative groups.

36 Primary and Secondary outcome measures: The primary outcome of major cardiovascular 37 events from baseline screening to June 2021 was defined as hospitalisation or death due to 38 acute myocardial infarction, cerebrovascular diseases, heart failure, or peripheral vascular 39 diseases. The association between proteinuria and major cardiovascular events was assessed 40 using a Cox proportional hazards model.

**Results:** Of the 88,744 participants, 8,775 (9.9%) and 5,498 (6.2%) had once-positive and repetitivelypositive proteinuria, respectively. During the follow-up period of 402,799 personyears (median 5.25 years), 660 cardiovascular events were observed, and the incidence rate was 1.64 per 1,000 person-years (95% confidence interval [CI] 1.52–1.77). Despite adjusting for major cardiovascular risk factors, we observed a higher cardiovascular incidence in the repetitively positive (hazard ratio 2.08, 95% CI 1.67–2.59) and once-positive groups (hazard ratio 1.36, 95% CI 1.07–1.72). We found similar associations for acute myocardial infarction, Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

48 cerebrovascular disease, heart failure, and peripheral artery disease.

**Conclusions:** Proteinuria is often repeatedly detected during annual renal screening in the 50 general population. Repetitive proteinuria is associated with a greater risk of major 51 cardiovascular events than single-time proteinuria.

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#### 67 INTRODUCTION

The prevalence of cardiovascular disease (CVD) remains as high as 9.3% in the United States (US), and increased CVD prevalence remains a worldwide health concern. [1] A similar trend has been observed in Japan, where heart disease and CVD are the leading causes of death. [2] It is estimated that approximately 135,000 CVD events were prevented in the US between 2011 and 2016, while approximately 170,000 CVD events increased in individuals aged 45– 64 years. [3] CVD risk screening for the middle-aged-to-older population is an important strategy in preventing CVD events.

Proteinuria is a well-established cardiovascular risk factor. [4-12] Proteinuria is semi-quantitatively detectable by a low-cost dipstick urine test, annually performed nationwide in Japan. [13] Proteinuria screening, typically performed using a single-time dipstick urine test, has been recommended only for high-risk populations according to the guidelines for costeffectiveness. [14] Single-time proteinuria has been used in most general health screening programs and epidemiological studies. [5-12,15-17] Hence, the clinical significance of repetitive proteinuria has not been discussed thoroughly. There is a population that undergoes multiple proteinuria screenings and has repetitive positive results; however, the difference in CVD risk between single and multiple proteinuria is unclear. Therefore, the clinical significance of the second and subsequent proteinuria screening also remains unclear.

Until recently, therapeutic interventions for proteinuria were mainly limited to reninangiotensin-aldosterone system inhibitors. However, in 2020, treatment with sodium-glucose co-transporter 2 (SGLT2) inhibitors was shown to be effective for both heart and kidney diseases, even in the non-diabetic population, as a new intervention added to reninangiotensin-aldosterone system inhibitors. [18,19] The advent of SGLT2 inhibitors has

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92 increased the importance of proteinuria screening in preventing CVD and chronic kidney93 disease.

95 Dipstick urine tests have been performed annually and mandatorily in Japan for the general 96 population aged 40–74 years. We were presented with a unique opportunity to assess the 97 association between annual renal screening and CVD risk using these data. We aimed to 98 investigate the association between repetitive proteinuria and cardiovascular events using 99 health screening data and medical claims records in Japan. Findings from this study fill an 100 important knowledge gap in considering effective renal function screening in CVD risk 101 management in the general population.

## 103 MATERIALS AND METHODS

## 104 Setting and participants

We obtained health screening results and medical claims data from one of the largest health insurers in Japan (a national sample of employees of civil engineering and construction companies). Using these databases, we observed the cardiovascular outcomes after renal function screening in the general population. In the Japanese universal health insurance system, all medical care details (diagnosis, procedures, and other clinical practices) are recorded in the medical claims data. Furthermore, all individuals >40 years of age are obligated to undergo annual health screening. The corresponding author had full access to all data and was responsible for data analysis.

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114 We included participants aged 40–74 years who underwent renal function screening at least 115 twice during the baseline period from April 2011 to March 2015 (Figure 1) from a 116 nationwide health screening cohort in Japan. We excluded the participants who underwent

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dialysis at the baseline and those who experienced the major adverse cardiovascular event(MACE) outcome (primary endpoint) within 6 months prior to the baseline.

## 120 Main exposures and covariates

Positive proteinuria was defined as proteinuria  $\geq 1+$  using a urine dipstick test. We categorized the main exposures based on at least two proteinuria results: repetitively positive (positive twice or more), once-positive, and all-negative. The covariates included age (continuous); sex (binary); estimated glomerular filtration rate (eGFR) (continuous); body mass index (BMI) (continuous); systolic blood pressure (SBP) (continuous); haemoglobin A1c (HbA1c) (continuous); low-density lipoprotein (LDL) cholesterol (continuous); history of stroke (yes, no); history of myocardial infarction (yes, no); use of antihypertensive (yes, no), antidiabetic (yes, no), or antihyperlipidemic (yes, no) drugs; smoking (yes, no); and alcohol intake (none,  $<20 \text{ g/day}, \geq 20 \text{ g/day}$ ).

### 131 Primary outcomes

The primary endpoint was the first MACE; a composite of acute myocardial infarction (AMI),
stroke, heart failure (HF), or peripheral vascular disease (PVD). We defined MACE as the
records of hospitalisations or deaths with relevant diagnosis codes defined based on the
International Classification of Diseases, 10th revision (AMI: I20-25, stroke: I60-69, HF: I50,
PVD: I70). [20-22] The follow-up period started from the baseline screening and ended in
June 2021 (Figure 1).

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# 139 Statistical analyses

Baseline characteristics are presented as means with standard deviations for continuous
 variables and percentages for categorical variables, according to the proteinuria exposure

Survival analysis was conducted to determine the associations between proteinuria groups and composite cardiovascular events using Cox regression models adjusted for confounders. We developed three models based on the adjusting factors: Model 1, adjusted for age, sex, and eGFR; Model 2, adjusted for HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, in addition to Model 1; and Model 3, adjusted for smoking and alcohol intake, in addition to Model 2. These covariates were selected based on the clinical knowledge and previous studies. [4-9,23] 

153 Secondary analysis

groups.

Subgroup and two sensitivity analyses were performed. Subgroup analyses were performed according to four cardiovascular risks: hypertension (yes/no), diabetes (yes/no), hyperlipidaemia (yes/no), and smoking (yes/no). Because missing values existed in these cardiovascular factors, we conducted complete case analyses to ensure that the same population was analysed in the four subgroup analyses. For the first sensitivity analysis, we categorized proteinuria 1+ and proteinuria  $\geq 2+$  and evaluated the association with MACE by excluding the effect of proteinuria  $\geq 2+$  on MACE because the participants with repetitive proteinuria were more likely to have proteinuria  $\geq 2+$ . The redefined categories were as follows: at least one positive result of  $\geq 2+$  (2+ positive group), at least two positive results of 1+ with no results  $\geq$ 2+ (repetitive 1+ positive group), one positive result of 1+ (once 1+ positive group), and all-positive or negative results (All-negative group). For the second sensitivity analysis, a multiple imputation approach with chained equations ("mi impute" command in Stata) was used to account for missing data. In total, 20 imputation datasets were

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used.

## 169 PATIENT AND PUBLIC INVOLVEMENT

Patients and/or the public were not involved in the design, or conduct, or reporting, ordissemination plans of this research.

# **RESULTS**

Of the 179,840 participants who underwent health screenings in the database, 90,752 (50.5%) were excluded for undertaking the health screening fewer than two times, 344 (0.2%) were excluded for having a history of cardiovascular diseases, and 88,744 who underwent kidney function screenings at least twice were analysed (Figure 2). No dialysis patient had received this screening. Of the 88,744 eligible participants, 8,775 (9.9%) and 5,498 (6.2%) had once-positive and repetitively positive proteinuria, respectively. Detailed demographics and characteristics of the participants are presented in Table 1. In summary, the average age was 51.6±7.9 years, and 25.341 (28.6%) patients were females. Baseline proteinuria was negative or trace for 78,453 (95.7%) patients, and 3,726 (4.3%) patients had baseline proteinuria  $\geq 1+$ . The prevalence of hypertension and dyslipidaemia was 16.1% and 9.7%, respectively. Additionally, the prevalence of current smokers was 27.8%, similar to the overall smoking prevalence in Japan. [24] Compared with the participants in the all-negative and once-positive groups, the participants in the repetitively positive group likely had common CVD risk factors, that is, male sex, high BMI, low eGFR, high blood pressure, high HbA1c, high smoking prevalence, and a history of CVD (Table 2). The distribution of proteinuria severity in the excluded participants was similar to that of the included participants (Table S1).

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# 194 Table 1. Demographic and clinical characteristics of the participants

	Total	All-negative group	Once-positive group	Repetitively positive group	p-value
N	88,744	74,471	8,775	5,498	
Age, year	51.6±7.9	51.7±7.9	50.7±7.8	52.4±8.0	< 0.001
Female, no. (%)	25,341 (28.6%)	22,944 (30.8%)	1,760 (20.1%)	637 (11.6%)	< 0.001
Body mass index, kg/m <sup>2</sup>	23.7±3.6	23.5±3.4	24.4±3.9	25.9±4.5	< 0.001
Estimated glomerular filtration rate, ml/min/1.73 m <sup>2</sup>	78.4±13.9	78.6±13.3	78.7±14.3	74.8±19.0	< 0.001
Baseline proteinuria,* no. (%)	<i>N</i>				
-	77,729 (87.9%)	69,361 (93.4%)	6,318 (72.0%)	2,050 (37.4%)	< 0.001
±	7,024 (7.9%)	4,864 ( 6.6%)	1,254 (14.3%)	906 (16.5%)	
1+	2,616 (3.0%)	0 ( 0.0%)	1,043 (11.9%)	1,573 (28.7%)	
2+	864 (1.0%)	0(0.0%)	142 (1.6%)	722 (13.2%)	
3+	246 (0.3%)	0 ( 0.0%)	14 (0.2%)	232 ( 4.2%)	
Systolic blood pressure, mmHg	124±17	123.5±16.4	126.7±17.3	132.2±18.9	< 0.001
Diastolic blood pressure, mmHg	77±12	76.5±11.8	79.1±12.6	82.7±12.7	< 0.001
Haemoglobin A1c, %	5.6±0.7	5.6±0.6	5.7±0.9	6.1±1.2	< 0.001
LDL cholesterol, mg/dl	128±32.1	128.3±31.9	128.3±33.1	127.1±33.7	0.023
Use of antihypertensive drugs, no. (%)	14,232 (16.1%)	10,452 (14.0%)	1,751 (20.0%)	2,029 (37.0%)	< 0.001
Use of antidiabetic drugs, no. (%)	4,368 (4.9%)	2,822 (3.8%)	624 (7.1%)	922 (16.8%)	< 0.001
Use of antihyperlipidemic drugs, no. (%)	8,612 (9.7%)	6,737 (9.1%)	880 (10.0%)	995 (18.1%)	< 0.001
Current smoking status, no. (%)	24,680 (27.8%)	19,520 (26.2%)	3,030 (34.6%)	2,130 (38.8%)	< 0.001
History of stroke, no. (%)	1,085 (1.3%)	817 (1.1%)	113 (1.3%)	155 (2.9%)	< 0.001
History of cardiovascular disease, no. (%)	1,996 (2.3%)	1,503 (2.1%)	234 (2.7%)	259 (4.8%)	<0.001

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196 Variables are presented as mean±standard deviation or n (%).

197 \*Baseline proteinuria represents urine test results at the beginning of the observation period,

198 which was defined as visit 1

199 Table 2. The hazard ratios for primary and secondary outcomes in the survival analyses

			Hazard ratio (95%	CI)
Outcomes	Model	All-negative group	Once-positive group	Repetitively positive group
MACE	Model 1*	Reference	1.51 (1.20–1.90)	2.72 (2.22–3.35)
	Model 2†	Reference	1.35 (1.07–1.71)	2.14 (1.72–2.67)
	Model 3‡	Reference	1.36 (1.07–1.72)	2.08 (1.67–2.59)
Secondary outcomes	No.			
Acute myocardial infarction	Model 3‡	Reference	1.21 (0.78–1.85)	1.85 (1.27–2.70)
Cerebrovascular disease	Model 3‡	Reference	1.28 (0.93–1.77)	1.94 (1.44–2.61)
Heart failure	Model 3‡	Reference	1.50 (1.05–2.14)	1.88 (1.33–2.68)
Peripheral artery disease	Model 3‡	Reference	1.46 (1.03–2.08)	1.78 (1.26–2.51)
Abbreviations: MACE, major adverse cardiovascular event; eGFR, estimated glomerular				

201 filtration rate; HbA1c, haemoglobin A1c; BMI, body mass index; SBP, systolic blood

- 2 202 pressure; LDL, low-density lipoprotein.
- 203 \*Model 1 was adjusted for age, sex, and eGFR.

204 †Model 2 was adjusted for HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs,
205 antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of
206 stroke, in addition to Model 1.

 $^{3}_{4}$  207  $\ddagger$  Model 3 was adjusted for smoking and alcohol intake in addition to Model 2.

6 208

209 During the follow-up period of 402,799 person-years (median 5.25 years, interquartile range

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3.92-5.67 years), 660 MACEs were observed, with an incidence rate of 1.64 (95%) confidence interval [CI] 1.52-1.77) per 1,000 person-years. Overall, 224 AMIs, 364 cerebrovascular diseases, 276 HFs, and 276 peripheral artery diseases were recorded during the observation period. A total of 24,522 participants were dropped due to the change in the insurance system before the end of the study period. The dropped out participants were older with higher rate of comorbidities (Table S2). The hazard ratio in the repetitively positive group was 2.72 (95% CI 2.22-3.35), which is higher than 1.51 (95% CI 1.20-1.90) in the once-positive proteinuria group in Model 1 (Table 2). In Model 2, which was additionally adjusted for comorbidities in Model 1, the hazard ratio in the repetitively positive group was 2.14 (95% CI 1.72–2.67). In Model 3, which was additionally adjusted for current smoking and alcohol intake, the hazard ratio in the repetitive positive group was 2.08 (95% CI 1.67-2.59).

We analysed the components of MACE as secondary outcomes. These incidence rates were 4.7 (95% CI 4.1–5.3), 7.4 (95% CI 6.6–8.2), 5.5 (95% CI 4.9–6.2), and 5.5 (95% CI 4.9–6.2) per 100,000 person-years for AMI, cerebrovascular disease, HF, and peripheral artery disease, respectively. The hazard ratios in the once-positive proteinuria and repetitively positive groups were respectively 1.21 (95% CI 0.78–1.85) and 1.85 (95% CI 1.27–2.70) for acute myocardial infarction, 1.28 (95% CI 0.93-1.77) and 1.94 (95% CI 1.44-2.61) for cerebrovascular disease, 1.50 (95% CI 1.05-2.14) and 1.88 (95% CI 1.33-2.68) for HF, and 1.46 (95% CI 1.03–2.08) and 1.78 (95% CI 1.26–2.51) for peripheral artery disease.

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In all the subgroups, the hazard ratio in the repetitively positive group was statistically
significantly higher than that in the all-negative (reference) and once-positive groups (Figure
Regardless of the cardiovascular risk factors, the repetitively positive group was more

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The sensitivity analysis confirmed that the repeated proteinuria of 1+, not including  $\geq$ 2+, had a stronger association with MACE than the single-time proteinuria of 1+ (Table 3). The hazard ratio in the repetitively 1+ positive group was 1.72 (95% CI 1.05–2.81), which was higher than 1.37 (95% CI 0.93–2.00) in the once 1+ positive group and similar to 1.89 (95% CI 1.29–2.77) in the 2+ positive group. The risk of CVD in the group with repeated 1+ urine protein was similar to that of the group with 2+ or higher urine protein. 

Table 3. Sensitivity analysis with redefining proteinuria categories 

	All- negative group*	Once 1+ positive group†	Repetitively 1+ positive group‡	2+ positive group§
Hazard ratio (95% CI)	reference	1.37 (0.93–2.00)	1.72 (1.05–2.81)	1.89 (1.29–2.77)

\*All-negative group was defined by all-positive or negative results, which included participants with normal urine test results. 

<sup>†</sup>Once the 1+ positive group was defined by one positive result of 1+. This group included participants with only 1+ proteinuria but no severe proteinuria.

The repetitive 1+ positive group was defined by at least two positive results of 1+ with no results  $\geq 2+$ . This group included participants with proteinuria  $\geq 1 +$  and without severe proteinuria. 

§The 2+ positive group was defined as having at least one positive result  $\geq 2+$ . This group included patients with severe proteinuria. 

We also confirmed that the effect of missing data was negligible on the result of the multiple imputation analysis. The hazard ratio in the once-positive and repetitively positive groups 

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were 1.29 (95% CI 1.03–1.64) and 2.01 (95% CI 1.61–2.50), respectively. The missing proportion was 1.42% for alcohol consumption, 2.57% for a history of CVD, and 2.57% for a history of stroke, whereas the others accounted for <0.5% (Table S3). The demographics and clinical characteristics of participants with missing data are described in Table S4. DISCUSSION We found that repetitive proteinuria in the screening results was associated with a higher risk of MACE and its composites, including AMI, stroke, HF, and PVD. To our knowledge, this study is the first to show the clinical significance of repeated dipstick urine tests and that repetitive proteinuria is associated with a higher risk of CVD outcomes. The dipstick urine test is a classical tool, although a more sophisticated assessment strategy potentially makes it a new cost-effective CVD risk screening tool. Dipstick urine tests are used for routine screening; thus, an important feature of the present study is that it was conducted on the general population. Similar to previous studies that evaluated single-time proteinuria, we observed an association between one-time proteinuria and cardiovascular outcomes. [5-12] Just as a dose-response relationship has been known for the association between the severity of proteinuria and CVD, a dose-response relationship was also observed between the times of proteinuria and CVD. [7,8,10-12] Further, this relationship was observed in the subgroup analysis, and the association between repetitive proteinuria and CVD was robust regardless of 

the cardiovascular risk factors. Albuminuria measurements, which can detect micro-albuminuria, may be preferable to urine dipstick tests, but they are more expensive and difficult to be implemented in a mass screening program. Our findings fit the current situation of the preventive strategy for cardiovascular events in the general population.

A possible explanation is that unfavourable CVD outcomes are mediated by the arteriosclerosis-related mechanisms of vascular endothelium dysfunction, low-grade 

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> inflammation, and plaque destabilisation. [3] Those mechanisms may be undeterminable by other clinical characteristics and demographics. Repetitive proteinuria may represent the duration of underlying arteriosclerosis and a CVD risk, which is undetermined by other clinical factors.

Two characteristics of the study database should be noted. Due to the nature of employee data, a certain number of dropouts is inevitable as retirement occurs. Since dropout would not be associated with the presence or severity of proteinuria, it would have little impact on the outcome. Further, we also analysed the data until dropout occurred, and the study design was less susceptible to dropout. Second, the number of urine tests depended on the participants, from a minimum of two to a maximum of four times. If all participants had undergone urine test screening a maximum of four times, some participants in the all-negative group would have been categorized in the once-positive or repetitively positive group. This misclassification weakens the association between proteinuria and MACE, and the association observed in this study remained significant even in a conservative analysis. Thus, the result can be robust for the number of variations in urine tests.

Apart from the causal limitation due to the observational study design, this study has some other limitations. First, although we defined outcome combined disease codes with hospitalisation and death, the misclassification and upcoding derived from medical receipts may exist. Second, the database was mainly composed of employees or their families in a specific industry, and 71.3% of the participants were males. Therefore, we must be cautious when applying this result to the general population. Third, half of the participants in the database were excluded from the selection process. Even though the backgrounds of the excluded participants were similar to those of the participants, a selection bias may exist.

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2 3 4	307	In conclusion, proteinuria is often detected in the general population through regular renal
5 6	308	screening with dipstick urine tests. A single episode of proteinuria was a cardiovascular risk
7 8	309	factor, although repeated episodes of proteinuria showed an even greater cardiovascular risk.
9 10 11	310	In the general population, kidney screening with repeated urine tests may help identify
12 13	311	populations at a high risk of CVD. We need to evaluate the impact of repeated proteinuria
14 15	312	screening, that is, whether the intervention of renal screening with repeated urinalysis reduces
16 17 18	313	CVD events. These results suggest the need to redesign renal function screening to address
19 20	314	CVD risk in the general population.
21 22	315	
23 24 25	316	ETHICS APPROVAL
26 27	317	This study was approved by the Kyoto University Institutional Review Board (IRB No.
28 29	318	R0817) and the IRB waived the need for informed consent.
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42 43	324	COMPETING INTERESTS
44 45 46	325	Dr. Ohnishi, Dr. Mori and Dr. Fukuma have no competing interest to declare.
47 48	326	
49 50	327	AUTHOR CONTRIBUTIONS
51 52	328	Tsuyoshi Ohnishi contributed to the concept of design, interpretation of the data, and drafting
55 55	329	of the manuscript. Yuichiro Mori contributed critically to the revision of the manuscript for
56 57	330	important intellectual content. Shingo Fukuma contributed to the concept of design, data
58 59 60	331	analysis, data interpretation, and critical revision of the manuscript for important intellectual

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23 24 25	341	
26 27	342	SUPPLEMENTAL MATERIAL
28 29 20	343	Tables S1–S4
30 31 32	344	
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3 4	413	FIGURE LEGENDS
5 6	414	
7 8 0	415	Figure 1. Study time course
9 10 11	416	*Visit 1 represents the first visit for health screening after April 1, 2014.
12 13	417	
14 15 16	418	Figure 2. Inclusion and exclusion processes
17 18	419	
19 20	420	Figure 3. Subgroup analyses by cardiovascular risk factors
21 22 23	421	*The white squares indicate the hazard ratios in the all-negative group (reference).
24 25	422	†The black dots with bars indicate the hazard ratios and 95% confidence intervals in the
26 27 28	423	once-positive and repetitive-positive groups.
28 29 30	424	Abbreviations: Neg., All-negative group: once, Once-positive group: Rep., Repetitively
31 32	425	positive group.
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Inclusion and exclusion processes

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#### 

# 1 Table S1. Demographic and clinical characteristics of the included and excluded

# 2 participants

Demographic and clinical characteristics		Included	Excluded
Ν		88,744	91,096
Age, year		51.6±7.9	53.6±8.8
Female, no.(%)		25,341 (28.6%)	35,151 (38.6%)
Body mass index, kg/m <sup>2</sup>		23.7±3.6	23.6±3.7
Estimated glomerular filtration rate, ml/min/1	.73		
m <sup>2</sup>		78.4±13.9	76.8±14.7
Baseline proteinuria, no. (%)			
	-	77,729 (87.9%)	79,132 (87.1%)
	±	7,024 (7.9%)	8,041 (8.8%)
	1+	2,616 (3.0%)	2,653 (2.9%)
	2+	864 (1.0%)	807 (0.9%)
	3+	246 (0.3%)	220 (0.2%)
Systolic blood pressure, mmHg		124±17	124.6±17.7
diastolic blood pressure, mmHg		77±12	76.7±12.1
Hemoglobin A1c, %		5.6±0.7	5.7±0.7
LDL cholesterol, mg/dl		128±32.1	125.5±32.2
Use of antihypertensive drugs, no. (%)		14,232 (16.1%)	16,930 (18.6%)
Use of antidiabetic drugs, no. (%)		4,368 (4.9%)	5,053 (5.6%)
Use of antihyperlipidemic drugs, no. (%)		8,612 (9.7%)	10,379 (11.4%)
Current smoking, no. (%)		24,680 (27.8%)	24,453 (26.9%)
History of stroke, no. (%)		1,085 (1.3%)	1,185 (1.5%)
History of cardiovascular disease, no. (%)		1,996 (2.3%)	2,242 (2.8%)

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2 3	h	Variables are presented as the mean standard deviation on $r(0/)$
4	3	variables are presented as the mean $\pm$ standard deviation of n (%).
$\begin{array}{c} 2\\ 3\\ 4\\ 5\\ 6\\ 7\\ 8\\ 9\\ 10\\ 11\\ 12\\ 13\\ 14\\ 15\\ 16\\ 17\\ 18\\ 19\\ 20\\ 21\\ 23\\ 24\\ 25\\ 26\\ 27\\ 28\\ 29\\ 30\\ 31\\ 32\\ 33\\ 34\\ 35\\ 36\\ 37\\ 38\\ 39\\ 40\\ 41\\ 42\\ 43\\ 44\\ 546\\ 47\\ 48\\ 49\\ 50\\ 51\\ 52\\ 53\\ 54\\ 55\\ 56\\ 57\\ 58\\ 59\end{array}$	3	Variables are presented as the mean±standard deviation or n (%).
60		

3 4	5	Table S2. Demographic
5 6		Demographic and c
/ 8 9		N
10 11		Age, year
12 13		Female, no.(%)
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49 50		Use of antihyperlipi
51 52		Current smoking, no
53 54		History of stroke, no
55 56 57		History of cardiovas
58 59	6	*Variables are presen
60		

#### phic and clinical characteristics of the participants by drop-out

Demographic and clinical characteristics		Drop-out (-)	Drop-out (+)
N		64,222	24,522
Age, year		49.3±6.6	57.8±7.8
Female, no.(%)		17,455 (27.1%)	7,886 (32.2%)
Body mass index, kg/m <sup>2</sup>		23.8±3.7	23.6±3.5
Estimated glomerular filtration rate, ml/min/	1.73		
m <sup>2</sup>		79.5±13.5	75.7±14.3
Baseline proteinuria, no. (%)			
	-	56,259 (87.9%)	21,470 (87.8%)
	±	5,185 (8.1%)	1,839 (7.5%)
	1+	1,847 (2.9%)	769 (3.1%)
	2+	571 (0.9%)	293 (1.2%)
	3+	150 (0.2%)	96 (0.4%)
Systolic blood pressure, mmHg		123.4±16.3	126.9±17.8
Diastolic blood pressure, mmHg		76.9±12.1	77.6±11.8
Hemoglobin A1c, %		5.7±0.7	5.6±0.8
LDL cholesterol, mg/dl		128.3±32.1	128.2±32.2
Use of antihypertensive drugs, no. (%)		8,158 (12.7%)	6,074 (24.8%)
Use of antidiabetic drugs, no. (%)		2,421 (3.8%)	1,947 (7.9%)
Use of antihyperlipidemic drugs, no. (%)		4,996 (7.8%)	3,616 (14.8%)
Current smoking, no. (%)		18,463 (28.8%)	6,217 (25.4%)
History of stroke, no. (%)		591 (0.9%)	494 (2.1%)
History of cardiovascular disease, no. (%)		1,093 (1.8%)	903 (3.7%)

nted as the mean $\pm$ SD or n (%).

1	-	8		
Variable	Missing	Observed	Total	Missing
				proportion
Hemoglobin A1c	433	88,311	88,744	0.49%
Body mass index	2	88,742	88,744	0.00%
Systolic blood pressure	6	88,738	88,744	0.01%
LDL cholesterol	9	88,735	88,744	0.01%
Use of antihypertensive drugs	100	88,644	88,744	0.11%
Use of antidiabetic drugs	100	88,644	88,744	0.11%
Use of antihyperlipidemic drugs	100	88,644	88,744	0.11%
History of stroke	2,283	86,461	88,744	2.57%
History of cardiovascular disease	2,284	86,460	88,744	2.57%
	110	88 632	88 744	0 13%

# 7 Table S3. The number of participants with missing variables

	Alcohol consumption	1,262	87,482	88,744	1.42%
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		N
Age, year	51.2±7.9	2,634
Female, no. (%)	406 (15.4%)	2,634
Body mass index, kg/m <sup>2</sup>	24.2±3.6	2,632
Estimated glomerular filtration rate, ml/min/1.73 m <sup>2</sup>	77.8±14.1	2,634
Baseline proteinuria, no. (%)		2,625
· · ·	2,189 (83.4%)	
±	324 (12.3%)	
1+	84 (3.2%)	
2+	23 (0.9%)	
3+	5 (0.2%)	
Systolic blood pressure, mmHg	122±16	2,628
diastolic blood pressure, mmHg	77±12	2,628
Hemoglobin A1c, %	5.6±0.7	2,201
LDL cholesterol, mg/dl	124±31	2,625
Use of antihypertensive drugs, no. (%)	455 (18.0%)	2,534
Use of antidiabetic drugs, no. (%)	136 (5.4%)	2,534
Use of antihyperlipidemic drugs, no. (%)	277 (10.9%)	2,534
Current smoking, no. (%)	747 (29.6%)	2,522
History of stroke, no. (%)	7 (2.0%)	351
History of cardio vascular disease, no. (%)	14 (4.0%)	350

; p (70)

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STROBE Statemen	nt—ch	ecklist of items that should be included in reports of observational studies	ght, inclu	023-0716	
	Item No.	Recommendation	ding fo	Ω Page Ω No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	r US	51 J	we conducted a cohort study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	nseigne eS <sup>y</sup> relati	uly 2023	Repetitive proteinuria is associated with a greater risk
Introduction			ed t		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	ht Superieu o'fext and o	wnloaded	the difference in CVD risk between single and multiple proteinuria was unclear
Objectives	3	State specific objectives, including any prespecified hypotheses	ır (ABES) . data mininç	from http:/	important knowledge gap in considering effective renal function screening in CVD
Methods			, ≥	<sup>b</sup> mj	
Study design	4	Present key elements of study design early in the paper	trai	ope	The study is a cohort study
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	nìfig, ai	n.bmj.o	Methods setting and participants section
Participants	6	( <i>a</i> ) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	nd <sup>o</sup> simi	om/ on	Setting and participants section
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed	lar tech	June 8	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	nblogie	, 2025 ;	Main exposure and covariates section
Data sources/	8*	For each variable of interest, give sources of data and details of methods of assessment	<b>.</b> ,	at A	Main exposure and covariates
measurement		(measurement). Describe comparability of assessment methods if there is more than one group		gen	section
Bias	9	Describe any efforts to address potential sources of bias	8	Сеп	Secondary analysis section
Study size	10	Explain how the study size was arrived at	9	3ibli	Results first paragraph
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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	023-07161 ht, finclud	Main exposure and covariates section
Statistical	12	(a) Describe all statistical methods, including those used to control for confounding	3 ol Iñg	Statistical Analysis section
methods		(b) Describe any methods used to examine subgroups and interactions	n <u>3</u>	Secondary Analysis section
		(c) Explain how missing data were addressed	<u> Senc</u>	Secondary analysis section
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed	Sei	Statistical analyses section
		( <u>e</u> ) Describe any sensitivity analyses	023 gne	Secondary analysis section
Results			. Do men vd to	
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	wnload It Super Fext ar	Result first and second paragraphs
		(b) Give reasons for non-participation at each stage	ed f ieu าดใช	Result first paragraphs
		(c) Consider use of a flow diagram	r (A lata	Figure2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	h http:// BES) . mining	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	₩up <del>3</del>	Supplemental tableS3
		(c) Cohort study—Summarise follow-up time (eg, average and total amount)	tra:	Results second paragraph
Outcome data	15*	Cohort study—Report numbers of outcome events or summary measures over time	n.b	Results second paragraph
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	wNA	Not applicable
		Cross-sectional study—Report numbers of outcome events or summary measures	NA B	Not applicable
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision		Results second paragraph and Table
		(eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	June 8 lar tech	2
		(b) Report category boundaries when continuous variables were categorized	, 20	Not applicable
		( <i>c</i> ) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	25 at / ogles.	Not applicable
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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	23-0716 ht, Thclu	Result fourth and fifth paragraph, Table 3
Discussion			13 o ding	
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Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	023. Do ginemei glated t	Discussion fifth paragraph
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# Risk of cardiovascular disease associated with repeated proteinuria across multiple kidney function screenings among the middle-aged and older general population in Japan: a retrospective cohort study

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1 Risk of cardiovascular disease associated with repeated proteinuria across multiple 2 kidney function screenings among the middle-aged and older general population in 3 Japan: a retrospective cohort study 4 Tsuyoshi Ohnishi 5 6 Department of Nephrology, Kasukabe Chuo General Hospital, Kasukabe, Japan 7 Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan 8 9 Yuichiro Mori Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan 10 11 12 Shingo Fukuma Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan 13 14 15 **ORCID**: Tsuyoshi Ohnishi 0000-0002-2120-9757 16 Address for Correspondence: Shingo Fukuma, M.D., Ph.D. 17 Human Health Sciences, Kyoto University Graduate School of Medicine 18 54 Shogoin-Kawahara, Sakyo, Kyoto, 606-8507 Japan 19 20 E-mail: fukuma.shingo.3m@kyoto-u.ac.jp 21 Tel: 81-75-366-7675 22 23 Word count: 2595

ABSTRACT

#### **Objectives:** We aimed to investigate the association between repetitive proteinuria and cardiovascular events among the middle-aged and older general Japanese population. **Design:** Retrospective cohort study Setting: We used repeated health screening results and medical claim data from one of the largest health insurers in Japan. Participants: Among the middle-aged and older participants (40-74 years, n=179,840), 90,752 were excluded for undergoing health screening fewer than two times and 344 were excluded for having a history of cardiovascular diseases; 88,744 who underwent kidney function screenings at least twice (from April 2011 to March 2015) were included in the analysis. Based on dipstick proteinuria test results, the participants were divided into 'Repetitively-positive' (positive twice or more [positive proteinuria was defined as $\geq 1+1$ ), 'Once-positive,' and 'All-negative' groups. Primary and secondary outcome measures: The primary outcome of major cardiovascular events from baseline screening to June 2021 was hospitalisation or death due to acute

myocardial infarction, cerebrovascular diseases, heart failure, or peripheral vascular diseases.
The association between proteinuria and major cardiovascular events was assessed using a Cox
proportional hazards model.

Results: Of the 88,744 participants, 8,775 (9.9%) and 5,498 (6.2%) had Once-positive and Repetitively-positive proteinuria, respectively. During the follow-up period of 402,799 personyears (median 5.25 years), 660 cardiovascular events were observed, with an incidence of 1.64 per 1,000 person-years (95% confidence interval [CI] 1.52–1.77). Despite adjusting for major cardiovascular risk factors, we observed a high incidence of cardiovascular events in the Repetitively-positive (hazard ratio 2.08, 95% CI 1.67–2.59) and Once-positive groups (hazard ratio 1.36, 95% CI 1.07–1.72). We found similar associations for acute myocardial infarction,

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49 cerebrovascular disease, heart failure, and peripheral vascular disease.

50 Conclusions: Proteinuria is often repeatedly detected during annual renal screening in the

51 general population. Repetitive proteinuria is a risk factor for major cardiovascular events.

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2 3	54	Strengths and limitations of this study
4 5	54	Su cligtus and minitations of this study
5 6 7	55	• The study had a large number of participants and a long observation period, enabling
7 8 0	56	evaluation of the risk of cardiovascular disease in the general population.
10 11	57	• Evaluation of urinary protein, as an indicator of exposure, could be conducted annually in
12 13	58	the general population.
14 15	59	• By using health check-up and insurance data, we accurately tracked the incidence of
16 17	60	cardiovascular disease outcomes.
19 20	61	• The semi-quantitative evaluation of urinary protein was a limitation of this study.
21 22	62	• Due to the retrospective study design, the potential impact of residual confounding
23 24	63	factors such as the duration of diabetes and the presence of glomerulonephritis cannot be
25 26 27	64	ruled out.
27 28 29	65	
30 31	66	Keywords
32 33	67	proteinuria cardiovascular diseases kidney disease risk
34 25	07	protenturia, cardiovascular diseases, kieliey disease, fisk
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70 INTRODUCTION

The prevalence of cardiovascular disease (CVD) remains as high as 9.3% in the United States (US), and increased prevalence of CVD remains a worldwide health concern [1]. A similar trend has been observed in Japan, where heart disease and CVD are the leading causes of death [2]. It is estimated that approximately 135,000 CVD events were prevented in the US between 2011 and 2016, while the number of CVD events has increased by approximately 170,000 in individuals aged 45–64 years [3]. Screening of the risk of CVD among the middle-aged-toolder population is an important strategy in preventing CVD events.

Proteinuria is a well-established risk factor of CVD [4-12]. Proteinuria is semi-quantitatively detectable by a low-cost dipstick urine test, which is performed annually and nationwide in Japan [13]. According to the Kidney Disease Improving Global Out- comes guidelines, screening for urine protein is recommended only for high-risk populations, and typically, a single urine test is performed from a cost-effectiveness standpoint [14,15]. A single measurement of proteinuria has been used in most general health screening programs and epidemiological studies [5-12, 16-18]. Hence, the clinical significance of repetitive proteinuria has not been thoroughly discussed. There is a population that undergoes multiple proteinuria screenings and has repetitive positive results; however, the difference in the risk of CVD between single and multiple proteinuria is unclear. Therefore, the clinical significance of the second and subsequent proteinuria screenings also remains unclear.

91 Until recently, therapeutic interventions for proteinuria were mainly limited to renin-92 angiotensin-aldosterone system inhibitors. However, in 2020, treatment with sodium-glucose 93 co-transporter 2 (SGLT2) inhibitors was shown to be effective for both heart and kidney 94 diseases, even in the non-diabetic population, and this intervention was added to renin-

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angiotensin-aldosterone system inhibitors [19, 20]. The advent of SGLT2 inhibitors has increased the importance of proteinuria screening in preventing CVD and chronic kidney disease.

Dipstick urine tests have been performed annually and mandatorily in Japan for the general population aged 40–74 years. These data presented a unique opportunity to assess the association between annual renal screening and the risk of CVD using these data. Therefore, this study aimed to investigate the association between repetitive proteinuria and cardiovascular events using health screening data and medical claims records in Japan. The findings from this study can fill an important knowledge gap in terms of effective renal function screening in risk management for CVD in the general population.

# 07 MATERIALS AND METHODS

### 108 Setting and participants

In this retrospective cohort study, we obtained health screening results and medical claims data from one of the largest health insurers in Japan (a national sample of employees of civil engineering and construction companies). Using these databases, we analysed the cardiovascular outcomes after renal function screening in the general population. In the Japanese universal health insurance system, all medical care details (diagnosis, procedures, and other clinical practices) are recorded in the medical claims data. Furthermore, all individuals aged >40 years are obligated to undergo annual health screening. The corresponding author had full access to all data and was responsible for data analysis.

We included participants aged 40–74 years from a nationwide health screening cohort in Japan;
 these participants underwent renal function screening at least twice during the baseline period

 from April 2011 to March 2015 (Figure S1). We excluded participants who underwent dialysis at the baseline and those who experienced any major adverse cardiovascular event (MACE) outcomes (primary endpoint) within 6 months before the baseline screening.

# Main exposures and covariates

Positive proteinuria was defined as proteinuria  $\geq 1 + using a urine dipstick test. We categorised$ the main exposures based on at least two proteinuria results and divided the patients into the following groups: 'Repetitively-positive' (positive twice or more), 'Once-positive' (positive only once), and 'All-negative' groups. The covariates included age (continuous); sex (binary); estimated glomerular filtration rate (eGFR) (continuous); body mass index (BMI) (continuous); systolic blood pressure (SBP) (continuous); haemoglobin A1c (HbA1c) (continuous); lowdensity lipoprotein (LDL) cholesterol (continuous); history of stroke (yes, no); history of myocardial infarction (yes, no); use of antihypertensive (yes, no), antidiabetic (yes, no), or antihyperlipidemic (yes, no) drugs; smoking (yes, no); and alcohol intake (none, <20 g/day,  $\geq 20 \text{ g/day}$ ).

### **Primary outcomes**

The primary endpoint was the first MACE; a composite of acute myocardial infarction (AMI), stroke, heart failure (HF), or peripheral vascular disease (PVD). We defined MACE based on the records of hospitalisations or deaths with relevant diagnosis codes defined based on the International Classification of Diseases, 10th revision (AMI: I20-25, stroke: I60-69, HF: I50, PVD: 170) [21-23]. The follow-up period started from the baseline screening and ended in June 2021 (Figure S1).

#### Statistical analyses

Baseline characteristics are presented as means with standard deviations for continuous variables and as percentages for categorical variables, according to the proteinuria exposure groups.

Survival analysis was conducted to determine the associations between proteinuria groups and composite cardiovascular events using Cox regression models adjusted for confounders. We developed three models based on the adjusting factors: Model 1, adjusted for age, sex, and eGFR; Model 2, adjusted for HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, in addition to Model 1; and Model 3, adjusted for smoking and alcohol intake, in addition to Model 2. These covariates were selected based on clinical knowledge and previous studies [4-9, 24].

#### Secondary analysis

Subgroup and three sensitivity analyses were performed. Subgroup analyses were performed according to four cardiovascular risks: hypertension (yes/no), diabetes mellitus (yes/no), hyperlipidaemia (yes/no), and smoking (yes/no). We further analysed a subgroup without any risk factor for CVD. As these cardiovascular factors had missing values, we conducted complete case analyses to ensure that the same population was analysed in the four subgroup analyses. For the first sensitivity analysis, we categorised proteinuria 1+ and proteinuria  $\geq 2+$ and evaluated the association with MACE by excluding the effect of proteinuria  $\geq 2+$  on MACE, as participants with repetitive proteinuria were more likely to have proteinuria  $\geq 2+$ . The redefined categories were as follows: at least one positive result of  $\geq 2+$  (2+ positive group), at least two positive results of 1+ with no results  $\geq$ 2+ (repetitive 1+ positive group), one positive result of 1+ (once 1+ positive group), and all negative results (All-negative group). For the

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second sensitivity analysis, a multiple imputation approach with chained equations ('mi impute' command in Stata) was used to account for missing data. In total, 20 imputation datasets were used. In the third sensitivity analysis, we restricted participants to those who had undergone three or more urine tests, to minimise the impact of the number of urine tests on the results.

Patient and public involvement 

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**RESULTS** 

Of the 179,840 participants who underwent health screenings in the database, 90,752 (50.5%) were excluded for undergoing health screening fewer than two times, 344 (0.2%) were excluded for having a history of cardiovascular diseases, and finally, 88,744 who underwent kidney function screenings at least twice were analysed (Figure 1). No dialysis patient had undergone this screening. Of the 88,744 eligible participants, 8,775 (9.9%) and 5,498 (6.2%) were in the Once-positive and Repetitively-positive groups, respectively. Detailed demographics and characteristics of the participants are presented in Table 1. To summarise these characteristics, the average age was 51.6±7.9 years, and 25,341 (28.6%) patients were females. Overall, 78,453 (95.7%) patients had negative or trace baseline proteinuria, while 3,726 (4.3%) patients had baseline proteinuria  $\geq 1+$ . The prevalence of hypertension and dyslipidaemia was 16.1% and 9.7%, respectively. Additionally, the proportion of current smokers was 27.8%, similar to the prevalence of overall smoking reporting in Japan [25]. Compared with participants in the All-negative and Once-positive groups, those in the Repetitively-positive group were more likely to have common risk factors for CVD, such as being male, high BMI, low eGFR, high blood pressure, high HbA1c level, high smoking Page 11 of 37 **BMJ** Open prevalence, and a history of CVD (Table 1). The distribution of proteinuria severity in the excluded participants was similar to that in the included participants (Table S1). to beet terien only 

Table 1. Demographic and clinical	characteristics of the p	participants	ncludi		
	Total	All-negative group	Once-positive group	Repetitively-positive	p-
			uses re	group	
N	88,744	74,471	8,775 8,775	5,498	
Age, years	51.6±7.9	51.7±7.9	50.7±7.8 te su	52.4±8.0	<
Female, no. (%)	25,341 (28.6%)	22,944 (30.8%)	and 1,760 (20.1%) d d d d	637 (11.6%)	<
Body mass index, kg/m <sup>2</sup>	23.7±3.6	23.5±3.4	24.4±3.9	25.9±4.5	<
Estimated glomerular filtration	78.4±13.9	78.6±13.3	78.7±14.3	74.8±19.0	<
rate, ml/min/1.73 m <sup>2</sup>			J traini		
Baseline proteinuria,* no. (%)			ing, an		
-	77,729 (87.9%)	69,361 (93.4%)	6,318 (72.0%)	2,050 (37.4%)	<
±	7,024 (7.9%)	4,864 (6.6%)	1,254 (14.3%) ह	906 (16.5%)	
1+	2,616 (3.0%)	0 (0.0%)	1,043 (11.9%)og	1,573 (28.7%)	
2+	864 (1.0%)	0 (0.0%)	142 (1.6%) <sup>-</sup>	722 (13.2%)	
3+	246 (0.3%)	0 (0.0%)	14 (0.2%)	232 (4.2%)	
Systolic blood pressure, mmHg	124±17	123.5±16.4	126.7±17.3	132.2±18.9	<

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1 2 3 4	Diastolic blood pressure, mmHg	77±12	76.5±11.8	ight, includ 79.1±12.6	82.7±12.7	<0.001
5 6	Haemoglobin A1c, %	5.6±0.7	5.6±0.6	ang for a 5.7±0.9 for a	6.1±1.2	< 0.001
7 8	LDL cholesterol, mg/dL	128±32.1	128.3±31.9	128.3±33.1 Eng	127.1±33.7	0.023
9 10 11	Use of antihypertensive drugs,	14,232 (16.1%)	10,452 (14.0%)	related 1,751 (20.0%)	2,029 (37.0%)	< 0.001
12 13	no. (%)			ownlo to text		
14 15	Use of antidiabetic drugs, no.	4,368 (4.9%)	2,822 (3.8%)	624 (7.1%) fand d	922 (16.8%)	< 0.001
16 17 18	(%)			ata mi		
19 20	Use of antihyperlipidemic drugs,	8,612 (9.7%)	6,737 (9.1%)	بي (10.0%) 880 (10.0%) 880 (10.0%)	995 (18.1%)	< 0.001
21 22	no. (%)			l traini		
23 24 25	Current smoking status, no. (%)	24,680 (27.8%)	19,520 (26.2%)	3,030 (34.6%) a	2,130 (38.8%)	< 0.001
26 27	History of stroke, no. (%)	1,085 (1.3%)	817 (1.1%)	113 (1.3%) d simil	155 (2.9%)	< 0.001
28 29	History of cardiovascular	1,996 (2.3%)	1,503 (2.1%)	234 (2.7%) ar tech	259 (4.8%)	< 0.001
30 31 32	disease, no. (%)			inologi		
33 199 34	Variables are presented as mean ±	standard deviation or n	(%).	ie at s. Age		
35 36 200	Abbreviation: LDL, low-density lip	poprotein.		ince Bi		
37 38 201 39 40	*Baseline proteinuria represents ur	ine test results at the be	eginning of the observation	period, which was define	d as visit 1	
41 42 43 44 45		For peer review only	- http://bmjopen.bmj.com/site/	about/guidelines.xhtml		12

During the follow-up period of 402,799 person-years (median 5.25 years, interquartile range 3.92–5.67 years), 660 MACEs were observed, with an incidence of 1.64 (95% confidence interval [CI] 1.52–1.77) per 1,000 person-years. Overall, 224 AMIs, 364 cerebrovascular diseases, 276 HFs, and 276 peripheral vascular diseases were recorded during the observation period. A total of 24,522 participants dropped out due to change in the insurance system before the end of the study period. The drop-out participants were older and likely had comorbidities (Table S2).

In Model 1 (Table 2), the hazard ratio was 1.51 (95% CI 1.20–1.90) for the Once-positive group and 2.72 (95% CI 2.22–3.35) for the Repetitively-positive group. In Model 2, which was additionally adjusted for comorbidities in Model 1, the hazard ratio was 2.14 (95% CI 1.72– 2.67) for the Repetitively-positive group. In Model 3, which was additionally adjusted for current smoking and alcohol intake, the hazard ratio was 2.08 (95% CI 1.67–2.59) for the Repetitively-positive group.

Table 2. Hazard ratios for MACE as a primary outcome in the survival analyses

Model	All-negative group	Once-positive group	Repetitively-positive group
Model 1*	Reference	1.51 (1.20–1.90)	2.72 (2.22–3.35)
Model 2†	Reference	1.35 (1.07–1.71)	2.14 (1.72–2.67)
Model 3‡	Reference	1.36 (1.07–1.72)	2.08 (1.67–2.59)

Abbreviations: MACE, major adverse cardiovascular event; eGFR, estimated glomerular
filtration rate; HbA1c, haemoglobin A1c; BMI, body mass index; SBP, systolic blood pressure;
LDL, low-density lipoprotein.

\*Model 1 was adjusted for age, sex, and eGFR.

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\*Model 2 was adjusted for HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs,
antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of
stroke, in addition to Model 1.

224 #Model 3 was adjusted for smoking and alcohol intake in addition to Model 2.

225

226 We analysed the components of MACE as secondary outcomes. The incidence was 4.7 (95% CI 4.1–5.3), 7.4 (95% CI 6.6–8.2), 5.5 (95% CI 4.9–6.2), and 5.5 (95% CI 4.9–6.2) per 100,000 227 228 person-years for AMI, cerebrovascular disease, HF, and peripheral vascular disease, 229 respectively (Table 3). The hazard ratio for the Once-positive and Repetitively-positive groups were as follows: 1.21 (95% CI 0.78-1.85) and 1.85 (95% CI 1.27-2.70) for AMI, 1.28 (95% 230 231 CI 0.93-1.77) and 1.94 (95% CI 1.44-2.61) for cerebrovascular disease, 1.50 (95% CI 1.05-232 2.14) and 1.88 (95% CI 1.33–2.68) for HF, and 1.46 (95% CI 1.03–2.08) and 1.78 (95% CI 1.26–2.51) for peripheral vascular disease, respectively (Table 3). 233

234

# Table 3. Hazard ratios for secondary outcomes in the survival analyses

37					
38		Secondary outcomes	All-negative	Once-positive group	Repetitively-positive
39					
40			group		group
41 42					
43		Acute myocardial	Reference	1.s21 (0.78–1.85)	1.85 (1.27–2.70)
44 45 46		infarction			
47 48		Cerebrovascular disease	Reference	1.28 (0.93–1.77)	1.94 (1.44–2.61)
49 50		Heart failure	Reference	1.50 (1.05–2.14)	1.88 (1.33–2.68)
51 52 53		Peripheral vascular	Reference	1.46 (1.03–2.08)	1.78 (1.26–2.51)
55 54 55		disease			
56 57	236	Abbreviations: eGFR, estin	nated glomerula	r filtration rate; HbA1c	, haemoglobin A1c; BMI,
58 59	237	body mass index; SBP, syst	olic blood press	ure; LDL, low-density li	poprotein.

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\*The model used was the same as Model 3 for the primary outcome, and was adjusted for age, sex, eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, smoking, and alcohol.

In all the subgroups, the hazard ratio was significantly higher in the Repetitively-positive group than in the All-negative (Figure 2). In the subgroup without any risk factor for CVD, the hazard ratio was 2.15 (95% CI 1.15-4.01) and 2.81 (95% CI 1.20-6.56) in the Once-positive and Repetitively-positive groups, respectively (Table S3). Regardless of the cardiovascular risk factors, repetitive proteinuria was a risk factor for CVD. 

In the sensitivity analysis of recategorised exposures, the hazard ratio was 1.37 (95% CI 0.93– 2.00), 1.72 (95% CI 1.05–2.81), and 1.89 (95% CI 1.29–2.77) in the Once 1+ positive, repetitively 1+ positive, and repetitively 2+ positive groups, respectively. Repeated proteinuria of 1+, not including proteinuria of >2+, was associated with MACE. This sensitivity analysis confirmed that the frequency of proteinuria, rather than the severity of proteinuria, was a risk factor for CVDs.

We also confirmed that the effect of missing data was negligible on the result of the multiple imputation analysis. The hazard ratio in the Once-positive and Repetitively-positive groups was 1.29 (95% CI 1.03-1.64) and 2.01 (95% CI 1.61-2.50), respectively. The missing proportion was 1.42% for alcohol consumption, 2.57% for a history of CVD, and 2.57% for a history of stroke, whereas the others accounted for <0.5% (Table S4). The demographics and clinical characteristics of participants with missing data are described in Table S5. 

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In the third sensitivity analysis, we confirmed that the results of primary analysis were independent of the definition of proteinuria and the number of urine tests. The hazard ratios were 1.46 (95% CI 1.14–1.87), 2.25 (95% CI 1.59–3.19), and 2.12 (95% CI 1.60–2.82) in the Single-positive, Two-positive, and Three or more-positive groups, respectively (Table S6)

**DISCUSSION** 

We found that repetitive proteinuria in the screening results was associated with a high risk of MACE and its composites, including AMI, stroke, HF, and PVD. To the best of our knowledge, this study is the first to show the clinical significance of repeated dipstick urine tests and report that repetitive proteinuria is a risk factor for CVDs. The dipstick urine test is a classical tool, although a more sophisticated assessment strategy can potentially make it a new cost-effective CVD risk screening tool. Dipstick urine tests are used for routine screening; thus, an important feature of the present study is that it was conducted on the general population. Similar to previous studies that evaluated proteinuria only once, we observed an association between 'Once-positive' proteinuria and cardiovascular outcomes [5-12] Similar to the dose-response relationship between the severity of proteinuria and the incidence of CVD, a dose-response relationship was observed between the frequency of proteinuria and CVD [7, 8, 10-12]. Further, this relationship was observed in the subgroup analysis, and the association between repetitive proteinuria and CVD was robust regardless of the cardiovascular risk factors. Albuminuria measurements, which can detect micro-albuminuria, may be preferable to urine dipstick tests, but they are more expensive and difficult to be implemented in a mass screening program. Our findings support the clinical significance of repeated urine dipstick tests to identify high-risk population for CVD in the general population.

A possible explanation for the association between CVD and proteinuria is that unfavourable Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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288 CVD outcomes are mediated by the arteriosclerosis-related mechanisms of vascular 289 endothelium dysfunction, low-grade inflammation, and plaque destabilisation [3]. Those 290 mechanisms may be undeterminable by other clinical characteristics and demographics. 291 Repetitive proteinuria may represent both the duration of underlying arteriosclerosis and a risk 292 of CVD, which is undetermined by other clinical factors.

Two characteristics of the study database should be noted. Due to the nature of employee data, a certain number of dropouts is inevitable as retirement occurs. Since drop-out would not be associated with the presence or severity of proteinuria, it would have little impact on the outcome. Further, we analysed the data until drop-out occurred, making the study design less susceptible to drop-out. Second, the number of urine tests depended on the participants, from a minimum of two to a maximum of four. If all participants had undergone urine test screening a maximum of four times, some participants in the All-negative group would have been categorised in the Once-positive or Repetitively-positive group. This misclassification weakens the association between proteinuria and MACE, and the association observed in this study remained significant even in a conservative analysis. Thus, our results are robust for the number of urine tests at baseline.

Apart from the causal limitation due to the observational study design, this study has some other limitations. First, although the outcome was defined based on a combination of disease codes with hospitalisation and death, misclassification and upcoding derived from medical receipts may have occurred. Second, the database was mainly composed of employees or their families in a specific industry, and 71.3% of the participants were males. Therefore, we must be cautious when applying these results to the general population. Third, half of the participants in the database were excluded from the selection process. Even though the backgrounds of the Page 19 of 37

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excluded participants were similar to those of the included participants, a selection bias may have existed. Finally, it is important to acknowledge the presence of potential residual confounding factors, such as glomerulonephritis or infectious and autoimmune diseases. 

In conclusion, proteinuria is often detected in the general population through regular renal screening with dipstick urine tests. Both single and repeated episodes of proteinuria were found to be risk factors for CVD, and a dose-response relationship was observed between the number of proteinuria episodes and the incidence of CVD. In the general population, kidney screening with repeated urine tests may help identify populations at a high risk of CVD. We need to evaluate the impact of repeated proteinuria screening, that is, whether renal screening with repeated urinalysis reduces the incidence of CVD events. These results suggest the need to redesign renal function screening strategy to address the risk of CVD in the general population. 

**ETHICS APPROVAL** 

This study was approved by the Kyoto University Institutional Review Board (IRB No.

R0817) who waived the need for informed consent. 

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We would like to thank the insurers and their members for providing us with their health insurance data.

#### **COMPETING INTERESTS**

Dr. Ohnishi, Dr. Mori and Dr. Fukuma have no competing interest to declare.

#### **AUTHOR CONTRIBUTIONS**

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338	Tsuyoshi Ohnishi contributed to the study design, interpretation of the data, and drafting of the
339	manuscript. Yuichiro Mori contributed critically to the revision of the manuscript for important
340	intellectual content. Shingo Fukuma contributed to the study design, data analysis, data
341	interpretation, and critical revision of the manuscript for important intellectual content.
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346	
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348	The data underlying this article are not shared due to the privacy policy of data providers.
349	
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2 3 4	432	FIGURE LEGENDS
5 6	433	
7 8	434	Figure 1. Inclusion and exclusion processes
9 10 11	435	
12 13	436	Figure 2. Subgroup analyses by cardiovascular risk factors
14 15 16	437	*The white squares indicate the hazard ratios in the All-negative group (reference).
17 18	438	†The black dots with bars indicate the hazard ratios and 95% confidence intervals in the Once-
19 20	439	positive and Repetitively-positive groups.
21 22 23	440	Abbreviations: Neg., All-negative group: Once, Once-positive group: Rep., Repetitively-
23 24 25	441	positive group.
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Inclusion and exclusion processes

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# 5 Table S1. Demographic and clinical characteristics of the included and excluded

# 6 participants

Demographic and clinical characteristics	Included	Excluded
	participants	participants
N	88,744	91,096
Age, year	51.6±7.9	53.6±8.8
Female, no. (%)	25,341 (28.6%)	35,151 (38.6%)
Body mass index, kg/m <sup>2</sup>	23.7±3.6	23.6±3.7
Estimated glomerular filtration rate, mL/min/1.73 m <sup>2</sup>	78.4±13.9	76.8±14.7
Baseline proteinuria, no. (%)		
-	77,729 (87.9%)	79,132 (87.1%)
±	7,024 (7.9%)	8,041 (8.8%)
1+	2,616 (3.0%)	2,653 (2.9%)
2+	864 (1.0%)	807 (0.9%)
3+	246 (0.3%)	220 (0.2%)
Systolic blood pressure, mmHg	124±17	124.6±17.7
diastolic blood pressure, mmHg	77±12	76.7±12.1
Hemoglobin A1c, %	5.6±0.7	5.7±0.7
LDL cholesterol, mg/dL	128±32.1	125.5±32.2
Use of antihypertensive drugs, no. (%)	14,232 (16.1%)	16,930 (18.6%)
Use of antidiabetic drugs, no. (%)	4,368 (4.9%)	5,053 (5.6%)
Use of antihyperlipidemic drugs, no. (%)	8,612 (9.7%)	10,379 (11.4%)
Current smoking, no. (%)	24,680 (27.8%)	24,453 (26.9%)
History of stroke, no. (%)	1,085 (1.3%)	1,185 (1.5%)
History of cardiovascular disease, no. (%)	1,996 (2.3%)	2,242 (2.8%)

7 Variables are presented as mean  $\pm$  standard deviation or n (%).

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# 8 Table S2. Demographic and clinical characteristics of the participants by drop-out

Demographic and clinical characteristics	Drop-out (-)	Drop-out (+)
N	64,222	24,522
Age, year	49.3±6.6	57.8±7.8
Female, no.(%)	17,455 (27.1%)	7,886 (32.2%)
Body mass index, kg/m <sup>2</sup>	23.8±3.7	23.6±3.5
Estimated glomerular filtration rate, mL/min/1.73	79.5±13.5	75.7±14.3
m <sup>2</sup>		
Baseline proteinuria, no. (%)		
	56,259 (87.9%)	21,470 (87.8%)
±	5,185 (8.1%)	1,839 (7.5%)
1+	1,847 (2.9%)	769 (3.1%)
2+	571 (0.9%)	293 (1.2%)
3+	150 (0.2%)	96 (0.4%)
Systolic blood pressure, mmHg	123.4±16.3	126.9±17.8
Diastolic blood pressure, mmHg	76.9±12.1	77.6±11.8
Hemoglobin A1c, %	5.7±0.7	5.6±0.8
LDL cholesterol, mg/dL	128.3±32.1	128.2±32.2
Use of antihypertensive drugs, no. (%)	8,158 (12.7%)	6,074 (24.8%)
Use of antidiabetic drugs, no. (%)	2,421 (3.8%)	1,947 (7.9%)
Use of antihyperlipidemic drugs, no. (%)	4,996 (7.8%)	3,616 (14.8%)
Current smoking, no. (%)	18,463 (28.8%)	6,217 (25.4%)
History of stroke, no. (%)	591 (0.9%)	494 (2.1%)
History of cardiovascular disease, no. (%)	1,093 (1.8%)	903 (3.7%)

\*Variables are presented as mean  $\pm$  standard deviation or n (%).

10	Table S3. Hazard ratios for MAC	CE among patients without major risk factors		
11	(hypertension, diabetes, hyperlipidaemia, and smoking) for CVD			
	Group	HR (95% CI)		
	All-negative	Reference		
	Once-positive	2.15 (1.15-4.01)		
	Repetitively-positives	2.81 (1.20-6.56)		
12	*Adjusted for age, sex, and eGFR	R, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive		
13	drugs, antidiabetic drugs, antihyper	rlipidemic drugs, history of myocardial infarction, and		
14	history of stroke, smoking, and alc	ohol intake.		
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Variable	Missing	Observed	Total	Missing
				proportion
Hemoglobin A1c	433	88,311	88,744	0.49%
Body mass index	2	88,742	88,744	0.00%
Systolic blood pressure	6	88,738	88,744	0.01%
LDL cholesterol	9	88,735	88,744	0.01%
Use of antihypertensive drugs	100	88,644	88,744	0.11%
Use of antidiabetic drugs	100	88,644	88,744	0.11%
Use of antihyperlipidemic drugs	100	88,644	88,744	0.11%
History of stroke	2,283	86,461	88,744	2.57%
History of cardiovascular disease	2,284	86,460	88,744	2.57%
Current smoking status	112	88,632	88,744	0.13%
Alcohol consumption	1,262	87,482	88,744	1.42%

# 16 Table S4. Number of participants with missing variables

		Ν
Age, year	51.2±7.9	2,634
Female, no. (%)	406 (15.4%)	2,634
Body mass index, kg/m <sup>2</sup>	24.2±3.6	2,632
Estimated glomerular filtration rate, mL/min/1.73 m <sup>2</sup>	77.8±14.1	2,634
Baseline proteinuria, no. (%)		2,625
- 0	2,189 (83.4%)	
±	324 (12.3%)	
1+	84 (3.2%)	
2+	23 (0.9%)	
3+	5 (0.2%)	
Systolic blood pressure, mmHg	122±16	2,628
diastolic blood pressure, mmHg	77±12	2,628
Hemoglobin A1c, %	5.6±0.7	2,201
LDL cholesterol, mg/dL	124±31	2,625
Use of antihypertensive drugs, no. (%)	455 (18.0%)	2,534

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Use of antihyperlipidemic drugs, no. (%)	277 (10.9%)	2,534
Current smoking, no. (%)	747 (29.6%)	2,522
History of stroke, no. (%)	7 (2.0%)	351
History of cardio vascular disease, no. (%)	14 (4.0%)	350
*Variables are presented as the mean $\pm$ standard dev	iation or n (%).	

21	Table S6. Hazard ratios for MACE in the survival analyses	
	Urine test results	HR (95% CI)
	All negative	Reference
	Single positive	1.46 (1.14–1.87)
	Two positives	2.25 (1.59–3.19)
	Three or more positives	2.12 (1.60–2.82)
22	*Adjusted for age, sex, and eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive	
23	drugs antidiabetic drugs antihyperlipidemic drugs history of myocardial infarction and	
25	arugs, antidiadette arugs, antiliyperinplaenne arugs, instory or mydeardiar infaretion, and	
24	history of stroke, smoking, and alcohol intake.	
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STROBE Statemen	nt—ch	ecklist of items that should be included in reports of observational studies	ght, inclu	2023-0716	
	Item No.	Recommendation	ding fo	Dage	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	r Use	<u>۲۲</u>	we conducted a cohort study
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Introduction			ed to		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	nt Superieu o'fext and o	wnloaded	the difference in CVD risk between single and multiple proteinuria was unclear
Objectives	3	State specific objectives, including any prespecified hypotheses	ır (ABES) . data mining	from http:/	important knowledge gap in considering effective renal function screening in CVD
Methods			,, ≥	bmj	
Study design	4	Present key elements of study design early in the paper	trai	ope	The study is a cohort study
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	າໄຕິg, ar	n.bmj.c	Methods setting and participants section
Participants	6	( <i>a</i> ) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	ıd <sup>c</sup> simi	om/ on	Setting and participants section
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed	lar tech	June 8	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	nblogi	, 2025 ;	Main exposure and covariates section
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	ÿ <sub>7</sub>	at Agen	Main exposure and covariates section
Bias	9	Describe any efforts to address potential sources of bias	8	ce E	Secondary analysis section
Study size	10	Explain how the study size was arrived at	9	Bibli	Results first paragraph
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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	023-07161 ht, finclue	Main exposure and covariates section
Statistical	12	(a) Describe all statistical methods, including those used to control for confounding	3 o   iffig	Statistical Analysis section
methods		(b) Describe any methods used to examine subgroups and interactions	<u> </u>	Secondary Analysis section
		(c) Explain how missing data were addressed	<u>та с</u>	Secondary analysis section
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed	Sei	Statistical analyses section
		( <u>e</u> ) Describe any sensitivity analyses	023 gne	Secondary analysis section
Results			. Do men ed to	
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	wnload It Super fêxt ar	Result first and second paragraphs
		(b) Give reasons for non-participation at each stage	ed f ieu nď d	Result first paragraphs
		(c) Consider use of a flow diagram	r (A lata	Figure2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	ı http:// BES) . mining	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	₩up <del>3</del> /	Supplemental tableS3
		(c) Cohort study—Summarise follow-up time (eg, average and total amount)	trai	Results second paragraph
Outcome data	15*	Cohort study—Report numbers of outcome events or summary measures over time	n.b	Results second paragraph
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	wNA	Not applicable
		Cross-sectional study—Report numbers of outcome events or summary measures		Not applicable
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision		Results second paragraph and Table
		(eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	June 8 lar tech	2
		(b) Report category boundaries when continuous variables were categorized	ngv.A 20	Not applicable
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	025 at <i>i</i> ogles.	Not applicable
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Other analyses	17	Report other analyses done-eg analyses of subgroups and interactions, and sensitivity analyses	123-0716 ht, Thelue	Result fourth and fifth paragraph, Table 3
Discussion			13 o ding	
Key results	18	Summarise key results with reference to study objectives	n 1014 3.1	Discussion first paragraph
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	July 2 Ęnsei uses re	Discussion fourth paragraph
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# Risk of cardiovascular disease associated with repeated proteinuria across annual kidney function screening among the middle-aged and older general population in Japan: a retrospective cohort study

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1 Risk of cardiovascular disease associated with repeated proteinuria across annual kidney 2 function screening among the middle-aged and older general population in Japan: a 3 retrospective cohort study 4 Tsuyoshi Ohnishi 5 6 Department of Nephrology, Kasukabe Chuo General Hospital, Kasukabe, Japan 7 Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan 8 9 Yuichiro Mori Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan 10 11 12 Shingo Fukuma Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan 13 14 15 **ORCID**: Tsuyoshi Ohnishi 0000-0002-2120-9757 16 Address for Correspondence: Shingo Fukuma, M.D., Ph.D. 17 Human Health Sciences, Kyoto University Graduate School of Medicine 18 54 Shogoin-Kawahara, Sakyo, Kyoto, 606-8507 Japan 19 20 E-mail: fukuma.shingo.3m@kyoto-u.ac.jp 21 Tel: 81-75-366-7675 22 23 Word count: 3245

ABSTRACT

#### **Objectives:** We aimed to investigate the association between repetitive proteinuria and cardiovascular events among the middle-aged and older general Japanese population. **Design:** Retrospective cohort study Setting: We used repeated health screening results and medical claim data from one of the largest health insurers in Japan. Participants: Among the middle-aged and older participants (40-74 years, n=179,840), 90,752 were excluded for undergoing health screening fewer than two times and 344 were excluded for having a history of cardiovascular diseases; 88,744 who underwent kidney function screenings at least twice (from April 2011 to March 2015) were included in the analysis. Based on dipstick proteinuria test results, the participants were divided into 'Repetitively-positive' (positive twice or more [positive proteinuria was defined as $\geq 1+1$ ), 'Once-positive,' and 'All-negative' groups. Primary and secondary outcome measures: The primary outcome of major cardiovascular events from baseline screening to June 2021 was hospitalisation or death due to acute

myocardial infarction, cerebrovascular diseases, heart failure, or peripheral vascular diseases.
The association between proteinuria and major cardiovascular events was assessed using a Cox
proportional hazards model.

Results: Of the 88,744 participants, 8,775 (9.9%) and 5,498 (6.2%) had Once-positive and Repetitively-positive proteinuria, respectively. During the follow-up period of 402,799 personyears (median 5.25 years), 660 cardiovascular events were observed, with an incidence of 1.64 per 1,000 person-years (95% confidence interval [CI] 1.52–1.77). Despite adjusting for major cardiovascular risk factors, we observed a high incidence of cardiovascular events in the Repetitively-positive (hazard ratio 2.08, 95% CI 1.67–2.59) and Once-positive groups (hazard ratio 1.36, 95% CI 1.07–1.72). We found similar associations for acute myocardial infarction,

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49 cerebrovascular disease, heart failure, and peripheral vascular disease.

50 Conclusions: Proteinuria is often repeatedly detected during annual renal screening in the

51 general population. Repetitive proteinuria is a risk factor for major cardiovascular events.

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2 3	54	Strengths and limitations of this study
4 5	54	Su cligtus and minitations of this study
5 6 7	55	• The study had a large number of participants and a long observation period, enabling
7 8 0	56	evaluation of the risk of cardiovascular disease in the general population.
10 11	57	• Evaluation of urinary protein, as an indicator of exposure, could be conducted annually in
12 13	58	the general population.
14 15	59	• By using health check-up and insurance data, we accurately tracked the incidence of
16 17	60	cardiovascular disease outcomes.
19 20	61	• The semi-quantitative evaluation of urinary protein was a limitation of this study.
21 22	62	• Due to the retrospective study design, the potential impact of residual confounding
23 24	63	factors such as the duration of diabetes and the presence of glomerulonephritis cannot be
25 26 27	64	ruled out.
27 28 29	65	
30 31	66	Keywords
32 33	67	proteinuria cardiovascular diseases kidney disease risk
34 25	07	protenturia, cardiovascular diseases, kieliey disease, fisk
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## 70 INTRODUCTION

The prevalence of cardiovascular disease (CVD) remains as high as 9.3% in the United States (US), and increased prevalence of CVD remains a worldwide health concern [1]. A similar trend has been observed in Japan, where heart disease and CVD are the leading causes of death [2]. It is estimated that approximately 135,000 CVD events were prevented in the US between 2011 and 2016, while the number of CVD events has increased by approximately 170,000 in individuals aged 45–64 years [3]. Screening of the risk of CVD among the middle-aged-toolder population is an important strategy in preventing CVD events.

Proteinuria is a well-established risk factor of CVD [4-12]. Proteinuria is semi-quantitatively detectable by a low-cost dipstick urine test, which is performed annually and nationwide in Japan [13]. According to the Kidney Disease Improving Global Outcomes guidelines, screening for urine protein is recommended only for high-risk populations, and typically, a single urine test is performed from a cost-effectiveness standpoint [14,15]. A single measurement of proteinuria has been used in most general health screening programs and epidemiological studies [5-12, 16-18]. Hence, the clinical significance of repetitive proteinuria has not been thoroughly discussed. There is a population that undergoes multiple proteinuria screenings and has repetitive positive results; however, the difference in the risk of CVD between single and multiple proteinuria is unclear. Therefore, the clinical significance of the second and subsequent proteinuria screenings also remains unclear.

91 Until recently, therapeutic interventions for proteinuria were mainly limited to renin-92 angiotensin-aldosterone system inhibitors. However, in 2020, treatment with sodium-glucose 93 co-transporter 2 (SGLT2) inhibitors was shown to be effective for both heart and kidney 94 diseases, even in the non-diabetic population, and this intervention was added to renin-

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angiotensin-aldosterone system inhibitors [19, 20]. The advent of SGLT2 inhibitors has increased the importance of proteinuria screening in preventing CVD and chronic kidney disease.

Dipstick urine tests have been performed annually and mandatorily in Japan for the general population aged 40–74 years. These data presented a unique opportunity to assess the association between annual renal screening and the risk of CVD using these data. Therefore, this study aimed to investigate the association between repetitive proteinuria and cardiovascular events using health screening data and medical claims records in Japan. The findings from this study can fill an important knowledge gap in terms of effective renal function screening in risk management for CVD in the general population.

# 07 MATERIALS AND METHODS

# 108 Setting and participants

In this retrospective cohort study, we obtained health screening results and medical claims data from one of the largest health insurers in Japan (a national sample of employees of civil engineering and construction companies). Using these databases, we analysed the cardiovascular outcomes after renal function screening in the general population. In the Japanese universal health insurance system, all medical care details (diagnosis, procedures, and other clinical practices) are recorded in the medical claims data. Furthermore, all individuals aged >40 years are obligated to undergo annual health screening. The corresponding author had full access to all data and was responsible for data analysis.

We included participants aged 40–74 years from a nationwide health screening cohort in Japan;
 these participants underwent renal function screening at least twice during the baseline period

 from April 2011 to March 2015 (Figure S1). We excluded participants who underwent dialysis at the baseline and those who experienced any major adverse cardiovascular event (MACE) outcomes (primary endpoint) within 6 months before the baseline screening.

# Main exposures and covariates

Positive proteinuria was defined as proteinuria  $\geq 1 + using a urine dipstick test. We categorised$ the main exposures based on at least two proteinuria results and divided the patients into the following groups: 'Repetitively-positive' (positive twice or more), 'Once-positive' (positive only once), and 'All-negative' groups. The covariates included age (continuous); sex (binary); estimated glomerular filtration rate (eGFR) (continuous); body mass index (BMI) (continuous); systolic blood pressure (SBP) (continuous); haemoglobin A1c (HbA1c) (continuous); lowdensity lipoprotein (LDL) cholesterol (continuous); history of stroke (yes, no); history of myocardial infarction (yes, no); use of antihypertensive (yes, no), antidiabetic (yes, no), or antihyperlipidemic (yes, no) drugs; smoking (yes, no); and alcohol intake (none, <20 g/day,  $\geq 20 \text{ g/day}$ ).

# **Primary outcomes**

The primary endpoint was the first MACE; a composite of acute myocardial infarction (AMI), stroke, heart failure (HF), or peripheral vascular disease (PVD). We defined MACE based on the records of hospitalisations or deaths with relevant diagnosis codes defined based on the International Classification of Diseases, 10th revision (AMI: I20-25, stroke: I60-69, HF: I50, PVD: 170) [21-23]. The follow-up period started from the baseline screening and ended in June 2021 (Figure S1).

#### Statistical analyses

Baseline characteristics are presented as means with standard deviations for continuous variables and as percentages for categorical variables, according to the proteinuria exposure groups.

Survival analysis was conducted to determine the associations between proteinuria groups and composite cardiovascular events using Cox regression models adjusted for confounders. We developed three models based on the adjusting factors: Model 1, adjusted for age, sex, and eGFR; Model 2, adjusted for HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, in addition to Model 1; and Model 3, adjusted for smoking and alcohol intake, in addition to Model 2. These covariates were selected based on clinical knowledge and previous studies [4-9, 24].

#### Secondary analysis

Subgroup and three sensitivity analyses were performed. Subgroup analyses were performed according to four cardiovascular risks: hypertension (yes/no), diabetes mellitus (yes/no), hyperlipidaemia (yes/no), and smoking (yes/no). We further analysed a subgroup without any risk factor for CVD. As these cardiovascular factors had missing values, we conducted complete case analyses to ensure that the same population was analysed in the four subgroup analyses. For the first sensitivity analysis, we categorised proteinuria 1+ and proteinuria  $\geq 2+$ and evaluated the association with MACE by excluding the effect of proteinuria  $\geq 2+$  on MACE, as participants with repetitive proteinuria were more likely to have proteinuria  $\geq 2+$ . The redefined categories were as follows: at least one positive result of  $\geq 2+$  (2+ positive group), at least two positive results of 1+ with no results  $\geq$ 2+ (repetitive 1+ positive group), one positive result of 1+ (once 1+ positive group), and all negative results (All-negative group). For the

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second sensitivity analysis, a multiple imputation approach with chained equations ('mi impute' command in Stata) was used to account for missing data. In total, 20 imputation datasets were used. In the third sensitivity analysis, we restricted participants to those who had undergone three or more urine tests, to minimise the impact of the number of urine tests on the results.

Patient and public involvement 

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**RESULTS** 

Of the 179,840 participants who underwent health screenings in the database, 90,752 (50.5%) were excluded for undergoing health screening fewer than two times, 344 (0.2%) were excluded for having a history of cardiovascular diseases, and finally, 88,744 who underwent kidney function screenings at least twice were analysed (Figure 1). No dialysis patient had undergone this screening. Of the 88,744 eligible participants, 8,775 (9.9%) and 5,498 (6.2%) were in the Once-positive and Repetitively-positive groups, respectively. Detailed demographics and characteristics of the participants are presented in Table 1. To summarise these characteristics, the average age was 51.6±7.9 years, and 25,341 (28.6%) patients were females. Overall, 78,453 (95.7%) patients had negative or trace baseline proteinuria, while 3,726 (4.3%) patients had baseline proteinuria  $\geq 1+$ . The prevalence of hypertension and dyslipidaemia was 16.1% and 9.7%, respectively. Additionally, the proportion of current smokers was 27.8%, similar to the prevalence of overall smoking reporting in Japan [25]. Compared with participants in the All-negative and Once-positive groups, those in the Repetitively-positive group were more likely to have common risk factors for CVD, such as being male, high BMI, low eGFR, high blood pressure, high HbA1c level, high smoking Page 11 of 37 **BMJ** Open prevalence, and a history of CVD (Table 1). The distribution of proteinuria severity in the excluded participants was similar to that in the included participants (Table S1). to beet terien only 

Table 1. Demographic and clinical	ncludi				
	Total	All-negative group	Once-positive group	Repetitively-positive	p-
			uses re	group	
N	88,744	74,471	8,775 8,775	5,498	
Age, years	51.6±7.9	51.7±7.9	50.7±7.8 to show	52.4±8.0	<
Female, no. (%)	25,341 (28.6%)	22,944 (30.8%)	and 1,760 (20.1%) d d d d	637 (11.6%)	<
Body mass index, kg/m <sup>2</sup>	23.7±3.6	23.5±3.4	24.4±3.9	25.9±4.5	<
Estimated glomerular filtration	78.4±13.9	78.6±13.3	78.7±14.3	74.8±19.0	<
rate, ml/min/1.73 m <sup>2</sup>			J traini		
Baseline proteinuria,* no. (%)			ing, an		
-	77,729 (87.9%)	69,361 (93.4%)	6,318 (72.0%)	2,050 (37.4%)	<
±	7,024 (7.9%)	4,864 (6.6%)	1,254 (14.3%) ह	906 (16.5%)	
1+	2,616 (3.0%)	0 (0.0%)	1,043 (11.9%)og	1,573 (28.7%)	
2+	864 (1.0%)	0 (0.0%)	142 (1.6%) <sup>-</sup>	722 (13.2%)	
3+	246 (0.3%)	0 (0.0%)	14 (0.2%)	232 (4.2%)	
Systolic blood pressure, mmHg	124±17	123.5±16.4	126.7±17.3	132.2±18.9	<

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1 2 3 4	Diastolic blood pressure, mmHg	77±12	76.5±11.8	ight, includ 79.1±12.6	82.7±12.7	<0.001
5 6	Haemoglobin A1c, %	5.6±0.7	5.6±0.6	ang for a 5.7±0.9 for a	6.1±1.2	< 0.001
7 8	LDL cholesterol, mg/dL	128±32.1	128.3±31.9	128.3±33.1 Eng	127.1±33.7	0.023
9 10 11	Use of antihypertensive drugs,	14,232 (16.1%)	10,452 (14.0%)	related 1,751 (20.0%)	2,029 (37.0%)	< 0.001
12 13	no. (%)			ownlo to text		
14 15	Use of antidiabetic drugs, no.	4,368 (4.9%)	2,822 (3.8%)	624 (7.1%) fand d	922 (16.8%)	< 0.001
16 17 18	(%)			ata mi		
19 20	Use of antihyperlipidemic drugs,	8,612 (9.7%)	6,737 (9.1%)	بي (10.0%) 880 (10.0%) 880 (10.0%)	995 (18.1%)	< 0.001
21 22	no. (%)			ul traini		
23 24 25	Current smoking status, no. (%)	24,680 (27.8%)	19,520 (26.2%)	3,030 (34.6%) a	2,130 (38.8%)	< 0.001
26 27	History of stroke, no. (%)	1,085 (1.3%)	817 (1.1%)	113 (1.3%) d simil	155 (2.9%)	< 0.001
28 29	History of cardiovascular	1,996 (2.3%)	1,503 (2.1%)	234 (2.7%) ar tech	259 (4.8%)	< 0.001
30 31 32	disease, no. (%)			inologi		
33 199 34	Variables are presented as mean ±	standard deviation or n	(%).	ie at s. Age		
35 36 200	Abbreviation: LDL, low-density lip	poprotein.		ince Bi		
37 38 201 39 40	*Baseline proteinuria represents ur	ine test results at the be	eginning of the observation	period, which was define	d as visit 1	
41 42 43 44 45		For peer review only	- http://bmjopen.bmj.com/site/	about/guidelines.xhtml		12

During the follow-up period of 402,799 person-years (median 5.25 years, interquartile range 3.92–5.67 years), 660 MACEs were observed, with an incidence of 1.64 (95% confidence interval [CI] 1.52–1.77) per 1,000 person-years. Overall, 224 AMIs, 364 cerebrovascular diseases, 276 HFs, and 276 peripheral vascular diseases were recorded during the observation period. A total of 24,522 participants dropped out due to change in the insurance system before the end of the study period. The drop-out participants were older and likely had comorbidities (Table S2).

In Model 1 (Table 2), the hazard ratio was 1.51 (95% CI 1.20–1.90) for the Once-positive group and 2.72 (95% CI 2.22–3.35) for the Repetitively-positive group. In Model 2, which was additionally adjusted for comorbidities in Model 1, the hazard ratio was 2.14 (95% CI 1.72– 2.67) for the Repetitively-positive group. In Model 3, which was additionally adjusted for current smoking and alcohol intake, the hazard ratio was 2.08 (95% CI 1.67–2.59) for the Repetitively-positive group.

Table 2. Hazard ratios for MACE as a primary outcome in the survival analyses

Model	All-negative group	Once-positive group	Repetitively-positive group
Model 1*	Reference	1.51 (1.20–1.90)	2.72 (2.22–3.35)
Model 2†	Reference	1.35 (1.07–1.71)	2.14 (1.72–2.67)
Model 3‡	Reference	1.36 (1.07–1.72)	2.08 (1.67–2.59)

Abbreviations: MACE, major adverse cardiovascular event; eGFR, estimated glomerular
filtration rate; HbA1c, haemoglobin A1c; BMI, body mass index; SBP, systolic blood pressure;
LDL, low-density lipoprotein.

\*Model 1 was adjusted for age, sex, and eGFR.

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\*Model 2 was adjusted for HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs,
antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of
stroke, in addition to Model 1.

224 #Model 3 was adjusted for smoking and alcohol intake in addition to Model 2.

225

226 We analysed the components of MACE as secondary outcomes. The incidence was 4.7 (95% CI 4.1–5.3), 7.4 (95% CI 6.6–8.2), 5.5 (95% CI 4.9–6.2), and 5.5 (95% CI 4.9–6.2) per 100,000 227 228 person-years for AMI, cerebrovascular disease, HF, and peripheral vascular disease, 229 respectively (Table 3). The hazard ratio for the Once-positive and Repetitively-positive groups were as follows: 1.21 (95% CI 0.78-1.85) and 1.85 (95% CI 1.27-2.70) for AMI, 1.28 (95% 230 231 CI 0.93-1.77) and 1.94 (95% CI 1.44-2.61) for cerebrovascular disease, 1.50 (95% CI 1.05-232 2.14) and 1.88 (95% CI 1.33–2.68) for HF, and 1.46 (95% CI 1.03–2.08) and 1.78 (95% CI 1.26–2.51) for peripheral vascular disease, respectively (Table 3). 233

234

# Table 3. Hazard ratios for secondary outcomes in the survival analyses

37					
38		Secondary outcomes	All-negative	Once-positive group	Repetitively-positive
39					
40			group		group
41 42					
43		Acute myocardial	Reference	1.s21 (0.78–1.85)	1.85 (1.27–2.70)
44 45 46		infarction			
47 48		Cerebrovascular disease	Reference	1.28 (0.93–1.77)	1.94 (1.44–2.61)
49 50		Heart failure	Reference	1.50 (1.05–2.14)	1.88 (1.33–2.68)
51 52 53		Peripheral vascular	Reference	1.46 (1.03–2.08)	1.78 (1.26–2.51)
55 54 55		disease			
56 57	236	Abbreviations: eGFR, estin	nated glomerula	r filtration rate; HbA1c	, haemoglobin A1c; BMI,
58 59	237	body mass index; SBP, syst	olic blood press	ure; LDL, low-density li	poprotein.

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\*The model used was the same as Model 3 for the primary outcome, and was adjusted for age, sex, eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, smoking, and alcohol.

In all the subgroups, the hazard ratio was significantly higher in the Repetitively-positive group than in the All-negative (Figure 2). In the subgroup without any risk factor for CVD, the hazard ratio was 2.15 (95% CI 1.15-4.01) and 2.81 (95% CI 1.20-6.56) in the Once-positive and Repetitively-positive groups, respectively (Table S3). Regardless of the cardiovascular risk factors, repetitive proteinuria was a risk factor for CVD. 

In the sensitivity analysis of recategorised exposures, the hazard ratio was 1.37 (95% CI 0.93– 2.00), 1.72 (95% CI 1.05–2.81), and 1.89 (95% CI 1.29–2.77) in the Once 1+ positive, repetitively 1+ positive, and repetitively 2+ positive groups, respectively. Repeated proteinuria of 1+, not including proteinuria of >2+, was associated with MACE. This sensitivity analysis confirmed that the frequency of proteinuria, rather than the severity of proteinuria, was a risk factor for CVDs.

We also confirmed that the effect of missing data was negligible on the result of the multiple imputation analysis. The hazard ratio in the Once-positive and Repetitively-positive groups was 1.29 (95% CI 1.03-1.64) and 2.01 (95% CI 1.61-2.50), respectively. The missing proportion was 1.42% for alcohol consumption, 2.57% for a history of CVD, and 2.57% for a history of stroke, whereas the others accounted for <0.5% (Table S4). The demographics and clinical characteristics of participants with missing data are described in Table S5. 

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In the third sensitivity analysis, we confirmed that the results of primary analysis were independent of the definition of proteinuria and the number of urine tests. The hazard ratios were 1.46 (95% CI 1.14–1.87), 2.25 (95% CI 1.59–3.19), and 2.12 (95% CI 1.60–2.82) in the Single-positive, Two-positive, and Three or more-positive groups, respectively (Table S6)

**DISCUSSION** 

We found that repetitive proteinuria in the screening results was associated with a high risk of MACE and its composites, including AMI, stroke, HF, and PVD. To the best of our knowledge, this study is the first to show the clinical significance of repeated dipstick urine tests and report that repetitive proteinuria is a risk factor for CVDs. The dipstick urine test is a classical tool, although a more sophisticated assessment strategy can potentially make it a new cost-effective CVD risk screening tool. Dipstick urine tests are used for routine screening; thus, an important feature of the present study is that it was conducted on the general population. Similar to previous studies that evaluated proteinuria only once, we observed an association between 'Once-positive' proteinuria and cardiovascular outcomes [5-12] Similar to the dose-response relationship between the severity of proteinuria and the incidence of CVD, a dose-response relationship was observed between the frequency of proteinuria and CVD [7, 8, 10-12]. Further, this relationship was observed in the subgroup analysis, and the association between repetitive proteinuria and CVD was robust regardless of the cardiovascular risk factors. Albuminuria measurements, which can detect micro-albuminuria, may be preferable to urine dipstick tests, but they are more expensive and difficult to be implemented in a mass screening program [26]. Our findings support the clinical significance of repeated urine dipstick tests to identify high-risk population for CVD in the general population.

> A possible explanation for the association between CVD and proteinuria is that unfavourable

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288 CVD outcomes are mediated by the arteriosclerosis-related mechanisms of vascular 289 endothelium dysfunction, low-grade inflammation, and plaque destabilisation [3]. Those 290 mechanisms may be undeterminable by other clinical characteristics and demographics. 291 Repetitive proteinuria may represent both the duration of underlying arteriosclerosis and a risk 292 of CVD, which is undetermined by other clinical factors.

Two characteristics of the study database should be noted. Due to the nature of employee data, a certain number of dropouts is inevitable as retirement occurs. Since drop-out would not be associated with the presence or severity of proteinuria, it would have little impact on the outcome. Further, we analysed the data until drop-out occurred, making the study design less susceptible to drop-out. Second, the number of urine tests depended on the participants, from a minimum of two to a maximum of four. If all participants had undergone urine test screening a maximum of four times, some participants in the All-negative group would have been categorised in the Once-positive or Repetitively-positive group. This misclassification weakens the association between proteinuria and MACE, and the association observed in this study remained significant even in a conservative analysis. Thus, our results are robust for the number of urine tests at baseline.

Apart from the causal limitation due to the observational study design, this study has some other limitations. First, although the outcome was defined based on a combination of disease codes with hospitalisation and death, misclassification and upcoding derived from medical receipts may have occurred. Second, the database was mainly composed of employees or their families in a specific industry, and 71.3% of the participants were males. Therefore, we must be cautious when applying these results to the general population. Third, half of the participants in the database were excluded from the selection process. Even though the backgrounds of the Page 19 of 37

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excluded participants were similar to those of the included participants, a selection bias may have existed. Finally, it is important to acknowledge the presence of potential residual confounding factors, such as glomerulonephritis or infectious and autoimmune diseases. 

In conclusion, proteinuria is often detected in the general population through regular renal screening with dipstick urine tests. Both single and repeated episodes of proteinuria were found to be risk factors for CVD, and a dose-response relationship was observed between the number of proteinuria episodes and the incidence of CVD. In the general population, kidney screening with repeated urine tests may help identify populations at a high risk of CVD. We need to evaluate the impact of repeated proteinuria screening, that is, whether renal screening with repeated urinalysis reduces the incidence of CVD events. These results suggest the need to redesign renal function screening strategy to address the risk of CVD in the general population. 

**ETHICS APPROVAL** 

This study was approved by the Kyoto University Institutional Review Board (IRB No.

R0817) who waived the need for informed consent. 

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We would like to thank the insurers and their members for providing us with their health insurance data.

#### **COMPETING INTERESTS**

Dr. Ohnishi, Dr. Mori and Dr. Fukuma have no competing interest to declare.

#### **AUTHOR CONTRIBUTIONS**

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338	Tsuyoshi Ohnishi contributed to the study design, interpretation of the data, and drafting of the
339	manuscript. Yuichiro Mori contributed critically to the revision of the manuscript for important
340	intellectual content. Shingo Fukuma contributed to the study design, data analysis, data
341	interpretation, and critical revision of the manuscript for important intellectual content.
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346	
347	DATA AVAILABILITY STATEMENT
348	The data underlying this article are not shared due to the privacy policy of data providers.
349	
350	SUPPLEMENTAL MATERIAL
351	Figure S1
352	Tables S1–S6
353	
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2 3 4	435	FIGURE LEGENDS
5 6	436	
7 8 9	437	Figure 1. Inclusion and exclusion processes
9 10 11	438	
12 13	439	Figure 2. Subgroup analyses by cardiovascular risk factors
14 15 16	440	*The white squares indicate the hazard ratios in the All-negative group (reference).
16 17 18	441	†The black dots with bars indicate the hazard ratios and 95% confidence intervals in the Once-
19 20	442	positive and Repetitively-positive groups.
21 22	443	Abbreviations: Neg., All-negative group: Once, Once-positive group: Rep., Repetitively-
23 24 25	444	positive group.
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	445	
53 54 55 56 57 58 59 60		







Inclusion and exclusion processes

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# 5 Table S1. Demographic and clinical characteristics of the included and excluded

# 6 participants

Demographic and clinical characteristics	Included	Excluded
	participants	participants
N	88,744	91,096
Age, year	51.6±7.9	53.6±8.8
Female, no. (%)	25,341 (28.6%)	35,151 (38.6%)
Body mass index, kg/m <sup>2</sup>	23.7±3.6	23.6±3.7
Estimated glomerular filtration rate, mL/min/1.73 m <sup>2</sup>	78.4±13.9	76.8±14.7
Baseline proteinuria, no. (%)		
-	77,729 (87.9%)	79,132 (87.1%)
±	7,024 (7.9%)	8,041 (8.8%)
1+	2,616 (3.0%)	2,653 (2.9%)
2+	864 (1.0%)	807 (0.9%)
3+	246 (0.3%)	220 (0.2%)
Systolic blood pressure, mmHg	124±17	124.6±17.7
diastolic blood pressure, mmHg	77±12	76.7±12.1
Hemoglobin A1c, %	5.6±0.7	5.7±0.7
LDL cholesterol, mg/dL	128±32.1	125.5±32.2
Use of antihypertensive drugs, no. (%)	14,232 (16.1%)	16,930 (18.6%)
Use of antidiabetic drugs, no. (%)	4,368 (4.9%)	5,053 (5.6%)
Use of antihyperlipidemic drugs, no. (%)	8,612 (9.7%)	10,379 (11.4%)
Current smoking, no. (%)	24,680 (27.8%)	24,453 (26.9%)
History of stroke, no. (%)	1,085 (1.3%)	1,185 (1.5%)
History of cardiovascular disease, no. (%)	1,996 (2.3%)	2,242 (2.8%)

7 Variables are presented as mean  $\pm$  standard deviation or n (%).

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# 8 Table S2. Demographic and clinical characteristics of the participants by drop-out

Demographic and clinical characteristics	Drop-out (-)	Drop-out (+)
N	64,222	24,522
Age, year	49.3±6.6	57.8±7.8
Female, no.(%)	17,455 (27.1%)	7,886 (32.2%)
Body mass index, kg/m <sup>2</sup>	23.8±3.7	23.6±3.5
Estimated glomerular filtration rate, mL/min/1.73	79.5±13.5	75.7±14.3
m <sup>2</sup>		
Baseline proteinuria, no. (%)		
	56,259 (87.9%)	21,470 (87.8%)
±	5,185 (8.1%)	1,839 (7.5%)
1+	1,847 (2.9%)	769 (3.1%)
2+	571 (0.9%)	293 (1.2%)
3+	150 (0.2%)	96 (0.4%)
Systolic blood pressure, mmHg	123.4±16.3	126.9±17.8
Diastolic blood pressure, mmHg	76.9±12.1	77.6±11.8
Hemoglobin A1c, %	5.7±0.7	5.6±0.8
LDL cholesterol, mg/dL	128.3±32.1	128.2±32.2
Use of antihypertensive drugs, no. (%)	8,158 (12.7%)	6,074 (24.8%)
Use of antidiabetic drugs, no. (%)	2,421 (3.8%)	1,947 (7.9%)
Use of antihyperlipidemic drugs, no. (%)	4,996 (7.8%)	3,616 (14.8%)
Current smoking, no. (%)	18,463 (28.8%)	6,217 (25.4%)
History of stroke, no. (%)	591 (0.9%)	494 (2.1%)
History of cardiovascular disease, no. (%)	1,093 (1.8%)	903 (3.7%)

\*Variables are presented as mean  $\pm$  standard deviation or n (%).

10	Table S3. Hazard ratios for MACE among patients without major risk fact					
11	(hypertension, diabetes, hyperlipidaemia, and smoking) for CVD					
	Group	HR (95% CI)				
	All-negative	Reference				
	Once-positive	2.15 (1.15-4.01)				
	Repetitively-positives	2.81 (1.20-6.56)				
12	*Adjusted for age, sex, and eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensiv					
13	drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and					
14	history of stroke, smoking, and alcohol intake.					
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Variable	Missing	Observed	Total	Missing
				proportion
Hemoglobin A1c	433	88,311	88,744	0.49%
Body mass index	2	88,742	88,744	0.00%
Systolic blood pressure	6	88,738	88,744	0.01%
LDL cholesterol	9	88,735	88,744	0.01%
Use of antihypertensive drugs	100	88,644	88,744	0.11%
Use of antidiabetic drugs	100	88,644	88,744	0.11%
Use of antihyperlipidemic drugs	100	88,644	88,744	0.11%
History of stroke	2,283	86,461	88,744	2.57%
History of cardiovascular disease	2,284	86,460	88,744	2.57%
Current smoking status	112	88,632	88,744	0.13%
Alcohol consumption	1,262	87,482	88,744	1.42%

# 16 Table S4. Number of participants with missing variables
		Ν
Age, year	51.2±7.9	2,634
Female, no. (%)	406 (15.4%)	2,634
Body mass index, kg/m <sup>2</sup>	24.2±3.6	2,632
Estimated glomerular filtration rate, mL/min/1.73 m <sup>2</sup>	77.8±14.1	2,634
Baseline proteinuria, no. (%)		2,625
- 0	2,189 (83.4%)	
±	324 (12.3%)	
1+	84 (3.2%)	
2+	23 (0.9%)	
3+	5 (0.2%)	
Systolic blood pressure, mmHg	122±16	2,628
diastolic blood pressure, mmHg	77±12	2,628
Hemoglobin A1c, %	5.6±0.7	2,201
LDL cholesterol, mg/dL	124±31	2,625
Use of antihypertensive drugs, no. (%)	455 (18.0%)	2,534

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Use of antihyperlipidemic drugs, no. (%)	277 (10.9%)	2,534
Current smoking, no. (%)	747 (29.6%)	2,522
History of stroke, no. (%)	7 (2.0%)	351
History of cardio vascular disease, no. (%)	14 (4.0%)	350
*Variables are presented as the mean $\pm$ standard dev	iation or n (%).	

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21	Table S6. Hazard ratios for MACE in the survival analyses			
	Urine test results	HR (95% CI)		
	All negative	Reference		
	Single positive	1.46 (1.14–1.87)		
	Two positives	2.25 (1.59–3.19)		
	Three or more positives	2.12 (1.60–2.82)		
22	*Adjusted for age, sex, and eGFR, HbA1c, BM	MI, SBP, LDL cholesterol, antihypertensive		
23	drugs antidiabetic drugs antihyperlipidemic dr	uss history of myocardial infarction and		
25		ugs, mistory of myocurdiar marchon, and		
24	history of stroke, smoking, and alcohol intake.			

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STROBE Statemen	nt—ch	ecklist of items that should be included in reports of observational studies	Jht, inclu	023-0716	
	Item No.	Recommendation	ding fo	α Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract		يت اگ	we conducted a cohort study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	nseigne eS¹relati	ıly 2023	Repetitive proteinuria is associated with a greater risk
Introduction			ed t	Do	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	nt Superieu o'fext and o	wnloaded	the difference in CVD risk between single and multiple proteinuria was unclear
Objectives	3	State specific objectives, including any prespecified hypotheses	ır (ABES) . data mining	from http://	important knowledge gap in considering effective renal function screening in CVD
Methods			, ≥	bmj	
Study design	4	Present key elements of study design early in the paper	trai	ope	The study is a cohort study
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	າໄຕິg, ar	n.bmj.c	Methods setting and participants section
Participants	6	( <i>a</i> ) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	ıd <sup>c</sup> simi	om/ on	Setting and participants section
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed	ar tech	June 8	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	nologie	, 2025 <b>;</b>	Main exposure and covariates section
Data sources/	8*	For each variable of interest, give sources of data and details of methods of assessment	ÿ7	At A	Main exposure and covariates
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Bias	9	Describe any efforts to address potential sources of bias	8	Се П	Secondary analysis section
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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	023-07161 ht, mclue	Main exposure and covariates section
Statistical	12	(a) Describe all statistical methods, including those used to control for confounding		Statistical Analysis section
methods		(b) Describe any methods used to examine subgroups and interactions	n <u>3</u>	Secondary Analysis section
		(c) Explain how missing data were addressed	<u> </u>	Secondary analysis section
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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	wnload It Supei fêxt ar	Result first and second paragraphs
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Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	ı http:// B€S) . mThing	Table 1
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		(c) Cohort study—Summarise follow-up time (eg, average and total amount)		Results second paragraph
Outcome data	15*	Cohort study—Report numbers of outcome events or summary measures over time	infi)	Results second paragraph
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	<del>نو</del> MA	Not applicable
		Cross-sectional study—Report numbers of outcome events or summary measures		Not applicable
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision		Results second paragraph and Table
		(eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	June 8 lar tech	2
		(b) Report category boundaries when continuous variables were categorized	nov/ 20	Not applicable
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	ogles.	Not applicable
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Other analyses	17	Report other analyses done-eg analyses of subgroups and interactions, and sensitivity analyses	ht, Thelue	Result fourth and fifth paragraph, Table 3
Discussion			ling	
Key results	18	Summarise key results with reference to study objectives	<b>í</b> 14	Discussion first paragraph
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	⊊nseij ⊊nseij uses re	Discussion fourth paragraph
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	gnemei slated t	Discussion fifth paragraph
Generalisability	21	Discuss the generalisability (external validity) of the study results	o tej	Discussion fifth paragraph
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Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	ieur (A Id data	Source of finding section
Note: An Explan checklist is best to http://www.anna	ation ised i ls.org	and Elaboration article discusses each checklist item and gives methodological background and published n conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmed /, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at w	example of the similar technologies.	y, Annals of Internal Medicine at re-statement.org.