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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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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 the largest 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 ≥1+] twice or more), 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 defined as 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 repetitivelypositive proteinuria, respectively. During the follow-up period of 402,799 person-years (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,

cerebrovascular disease, heart failure, and peripheral artery disease.

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

Strengths and limitations of this study

- This study is the first to show the clinical significance of repeated dipstick urine tests to identify a high risk of cardiovascular outcomes.
- We found associations between repetitive proteinuria and a higher risk for cardiovascular outcomes, providing a rationale for monitoring repeated episodes of proteinuria in kidney function screenings.
- We must be cautious while applying the study result to the general population since approximately 70% of the participants were male, and all participants were Japanese.

Keywords

repetitive proteinuria, cardiovascular diseases, kidney disease, general population, health screening

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 cost-effectiveness. [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 renin-angiotensin-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 renin-angiotensin-aldosterone system inhibitors. [18,19] 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. We were presented with a unique opportunity to assess the association between annual renal screening and CVD risk using these data. We aimed to investigate the association between repetitive proteinuria and cardiovascular events using health screening data and medical claims records in Japan. Findings from this study fill an important knowledge gap in considering effective renal function screening in CVD risk management in the general population.

MATERIALS AND METHODS

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.

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

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

Main exposures and covariates

Positive proteinuria was defined as proteinuria ≥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 g/day, ≥20 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 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).

Statistical analyses

Baseline characteristics are presented as means with standard deviations for continuous variables and 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 the clinical knowledge and previous studies. [4-9,23]

Secondary analysis

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 ≥2+ and evaluated the association with MACE by excluding the effect of proteinuria ≥2+ on MACE because the participants with repetitive proteinuria were more likely to have proteinuria ≥2+. The redefined categories were as follows: at least one positive result of ≥2+ (2+ positive group), at least two positive results of 1+ with no results ≥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

used.

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 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 ≥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^2	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^2	78.4±13.9	78.6±13.3	78.7±14.3	74.8±19.0	<0.001
Baseline proteinuria,* no. (%)					
-	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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Variables are presented as mean±standard deviation or n (%).

*Baseline proteinuria represents urine test results at the beginning of the observation period, which was defined as visit 1

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				
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 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.

†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.

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

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 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.

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 3). Regardless of the cardiovascular risk factors, the repetitively positive group was more

strongly associated with MACE than the once-positive group.

The sensitivity analysis confirmed that the repeated proteinuria of 1+, not including ≥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.

†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 ≥2+. This group included participants with proteinuria ≥ 1 + and without severe proteinuria.

§The 2+ positive group was defined as having at least one positive result ≥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

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

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.

In conclusion, proteinuria is often detected in the general population through regular renal screening with dipstick urine tests. A single episode of proteinuria was a cardiovascular risk factor, although repeated episodes of proteinuria showed an even greater cardiovascular risk. 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 the intervention of renal screening with repeated urinalysis reduces CVD events. These results suggest the need to redesign renal function screening to address CVD risk in the general population.

ETHICS APPROVAL

This study was approved by the Kyoto University Institutional Review Board (IRB No. R0817) and the IRB waived the need for informed consent.

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COMPETING INTERESTS

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

AUTHOR CONTRIBUTIONS

Tsuyoshi Ohnishi contributed to the concept of design, interpretation of the data, and drafting of the manuscript. Yuichiro Mori contributed critically to the revision of the manuscript for important intellectual content. Shingo Fukuma contributed to the concept of design, data analysis, data interpretation, and critical revision of the manuscript for important intellectual

content.

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DATA AVAILABILITY STATEMENT

The data underlying this article is not shared due to the privacy policy of data providers.

SUPPLEMENTAL MATERIAL

Tables S1–S4

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FIGURE LEGENDS

Figure 1. Study time course

*Visit 1 represents the first visit for health screening after April 1, 2014.

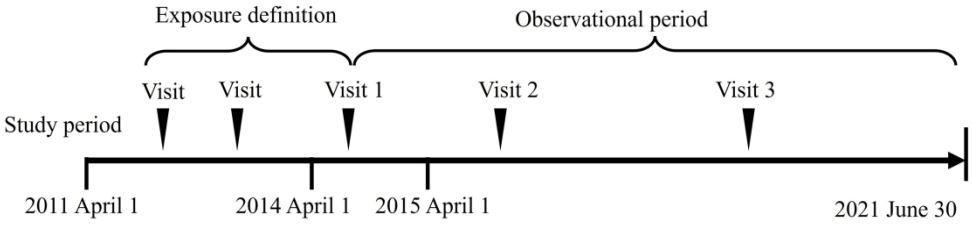
Figure 2. Inclusion and exclusion processes

Figure 3. Subgroup analyses by cardiovascular risk factors

*The white squares indicate the hazard ratios in the all-negative group (reference).

†The black dots with bars indicate the hazard ratios and 95% confidence intervals in the once-positive and repetitive-positive groups.

Abbreviations: Neg., All-negative group: once, Once-positive group: Rep., Repetitively positive group.



Study time course
*Visit 1 represents the first visit for health screening after April 1, 2014.

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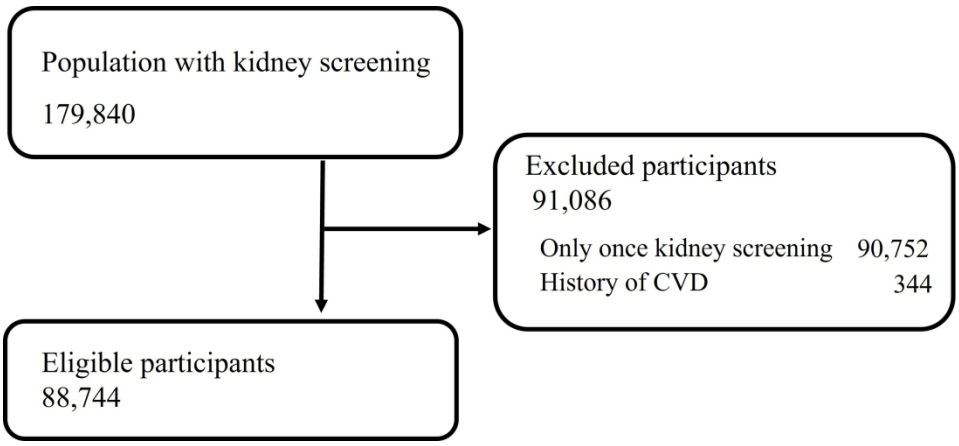

Inclusion and exclusion processes

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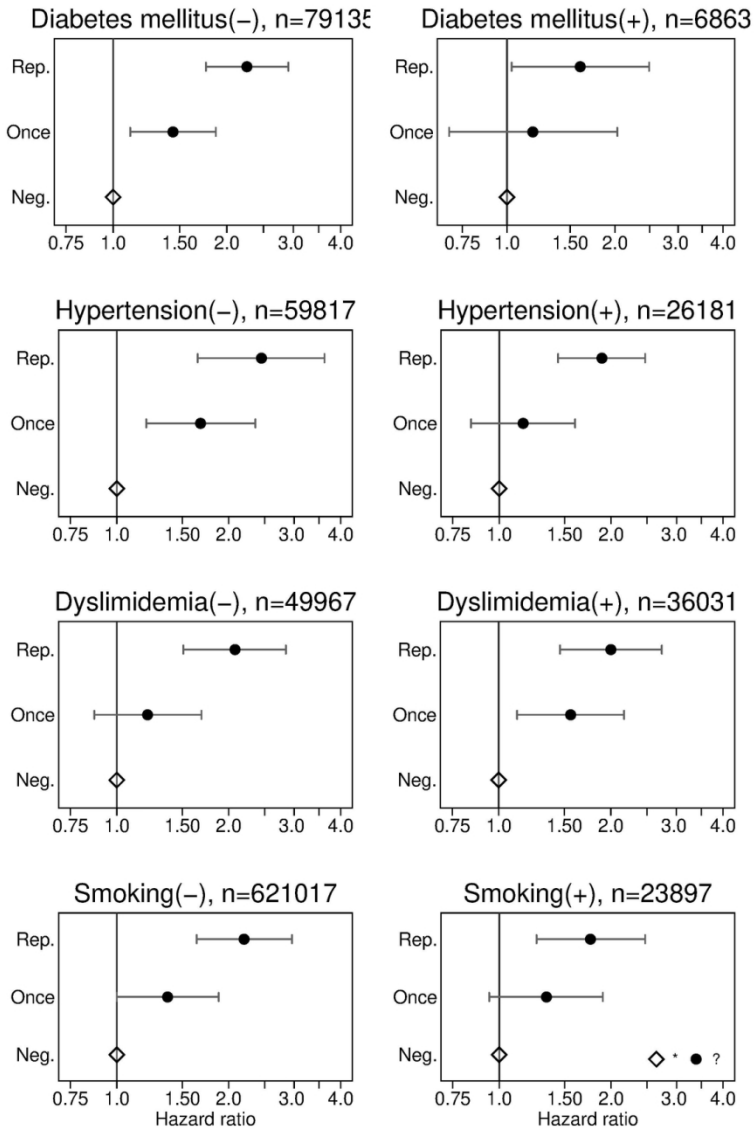

Subgroup analyses by cardiovascular risk factors
*The white squares indicate the hazard ratios in the all-negative group (reference).
†The black dots with bars indicate the hazard ratios and 95% confidence intervals in the once-positive and repetitive-positive groups.
Abbreviations: Neg., All-negative group: once, Once-positive group: Rep., Repetitively positive group.

101x152mm (300 x 300 DPI)

Table S1. Demographic and clinical characteristics of the included and excluded participants

Demographic and clinical characteristics		Included	Excluded
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^2		23.7±3.6	23.6±3.7
Estimated glomerular filtration rate, ml/min/1.73 m^2		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%)

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

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^2		23.8±3.7	23.6±3.5
Estimated glomerular filtration rate, ml/min/1.73 m^2		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%)

*Variables are presented as the mean±SD or n (%).

Table S3. The number of participants with missing variables

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%

Table S4. Demographic and clinical characteristics of participants who had missing data

			N
Age, year		51.2±7.9	2,634
Female, no. (%)		406 (15.4%)	2,634
Body mass index, kg/m^2		24.2±3.6	2,632
Estimated glomerular filtration rate, ml/min/1.73 m^2		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

*Variables are presented as the mean±SD or n (%).

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	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		we conducted a cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found		Repetitive proteinuria is associated with a greater risk
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported		the difference in CVD risk between single and multiple proteinuria was unclear
Objectives	3	State specific objectives, including any prespecified hypotheses		important knowledge gap in considering effective renal function screening in CVD
Methods				
Study design	4	Present key elements of study design early in the paper	5	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	5	Methods setting and participants section
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5	Setting and participants section
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed		Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7	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	7	Main exposure and covariates section
Bias	9	Describe any efforts to address potential sources of bias	8	Secondary analysis section
Study size	10	Explain how the study size was arrived at	9	Results first paragraph

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8	Main exposure and covariates section
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8	Statistical Analysis section
		(*b*) Describe any methods used to examine subgroups and interactions	8	Secondary Analysis section
		(*c*) Explain how missing data were addressed	8	Secondary analysis section
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	8	Statistical analyses section
		(*e*) Describe any sensitivity analyses	8	Secondary analysis section
Results				
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	8	Result first and second paragraphs
		(b) Give reasons for non-participation at each stage	8	Result first paragraphs
		(c) Consider use of a flow diagram	8	Figure2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	8	Supplemental tableS3
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8	Results second paragraph
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8	Results second paragraph
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*	*Not applicable*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*	*Not applicable*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11	Results second paragraph and Table 2
		(*b*) Report category boundaries when continuous variables were categorized	*NA*	*Not applicable*
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	*NA*	*Not applicable*

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12	Result fourth and fifth paragraph, Table 3
Discussion				
Key results	18	Summarise key results with reference to study objectives	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		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		Discussion fifth paragraph
Generalisability	21	Discuss the generalisability (external validity) of the study results		Discussion fifth paragraph
Other information				
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		Source of finding section

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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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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Primary Subject Heading:	Epidemiology
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Keywords:	NEPHROLOGY, PUBLIC HEALTH, EPIDEMIOLOGY, Cardiac Epidemiology < CARDIOLOGY

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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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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 ≥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 person-years (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,

cerebrovascular disease, heart failure, and peripheral vascular disease.

Conclusions: Proteinuria is often repeatedly detected during annual renal screening in the general population. Repetitive proteinuria is a risk factor for major cardiovascular events.

Strengths and limitations of this study

- The study had a large number of participants and a long observation period, enabling evaluation of the risk of cardiovascular disease in the general population.
- Evaluation of urinary protein, as an indicator of exposure, could be conducted annually in the general population.
- By using health check-up and insurance data, we accurately tracked the incidence of cardiovascular disease outcomes.
- The semi-quantitative evaluation of urinary protein was a limitation of this study.
- Due to the retrospective study design, the potential impact of residual confounding factors such as the duration of diabetes and the presence of glomerulonephritis cannot be ruled out.

Keywords

proteinuria, cardiovascular diseases, kidney disease, risk

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-to-older 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.

Until recently, therapeutic interventions for proteinuria were mainly limited to renin-angiotensin-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, and this intervention was added to renin-

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.

MATERIALS AND METHODS

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 ≥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); 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 g/day, ≥20 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: I70) [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 ≥2+ and evaluated the association with MACE by excluding the effect of proteinuria ≥2+ on MACE, as participants with repetitive proteinuria were more likely to have proteinuria ≥2+. The redefined categories were as follows: at least one positive result of ≥2+ (2+ positive group), at least two positive results of 1+ with no results ≥2+ (repetitive 1+ positive group), one positive result of 1+ (once 1+ positive group), and all 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 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 ≥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

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).

198 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, years	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^2	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^2	78.4±13.9	78.6±13.3	78.7±14.3	74.8±19.0	<0.001
Baseline proteinuria,* no. (%)					
-	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

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

Abbreviation: LDL, low-density lipoprotein.

*Baseline proteinuria represents urine test results at the beginning of the observation period, which was defined as visit 1

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.

†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.

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

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 person-years for AMI, cerebrovascular disease, HF, and peripheral vascular disease, 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% 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 vascular disease, respectively (Table 3).

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

Secondary outcomes	All-negative group	Once-positive group	Repetitively-positive group
Acute myocardial infarction	Reference	1.s21 (0.78–1.85)	1.85 (1.27–2.70)
Cerebrovascular disease	Reference	1.28 (0.93–1.77)	1.94 (1.44–2.61)
Heart failure	Reference	1.50 (1.05–2.14)	1.88 (1.33–2.68)
Peripheral vascular disease	Reference	1.46 (1.03–2.08)	1.78 (1.26–2.51)

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

*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.

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

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

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.

ACKNOWLEDGEMENTS

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

Tsuyoshi Ohnishi contributed to the study design, interpretation of the data, and drafting of the manuscript. Yuichiro Mori contributed critically to the revision of the manuscript for important intellectual content. Shingo Fukuma contributed to the study design, data analysis, data interpretation, and critical revision of the manuscript for important intellectual content.

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DATA AVAILABILITY STATEMENT

The data underlying this article are not shared due to the privacy policy of data providers.

SUPPLEMENTAL MATERIAL

Figure S1

Tables S1–S6

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FIGURE LEGENDS

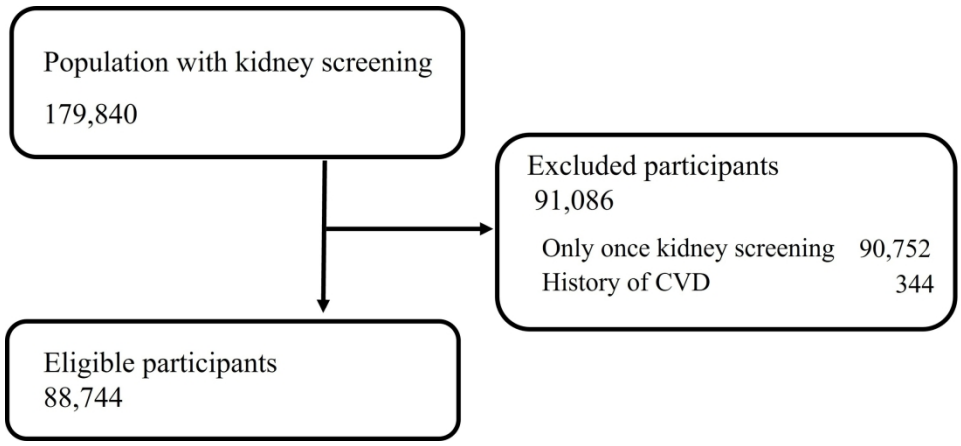
Figure 1. Inclusion and exclusion processes

Figure 2. Subgroup analyses by cardiovascular risk factors

*The white squares indicate the hazard ratios in the All-negative group (reference).

†The black dots with bars indicate the hazard ratios and 95% confidence intervals in the Once-positive and Repetitively-positive groups.

Abbreviations: Neg., All-negative group: Once, Once-positive group: Rep., Repetitively-positive group.



Inclusion and exclusion processes

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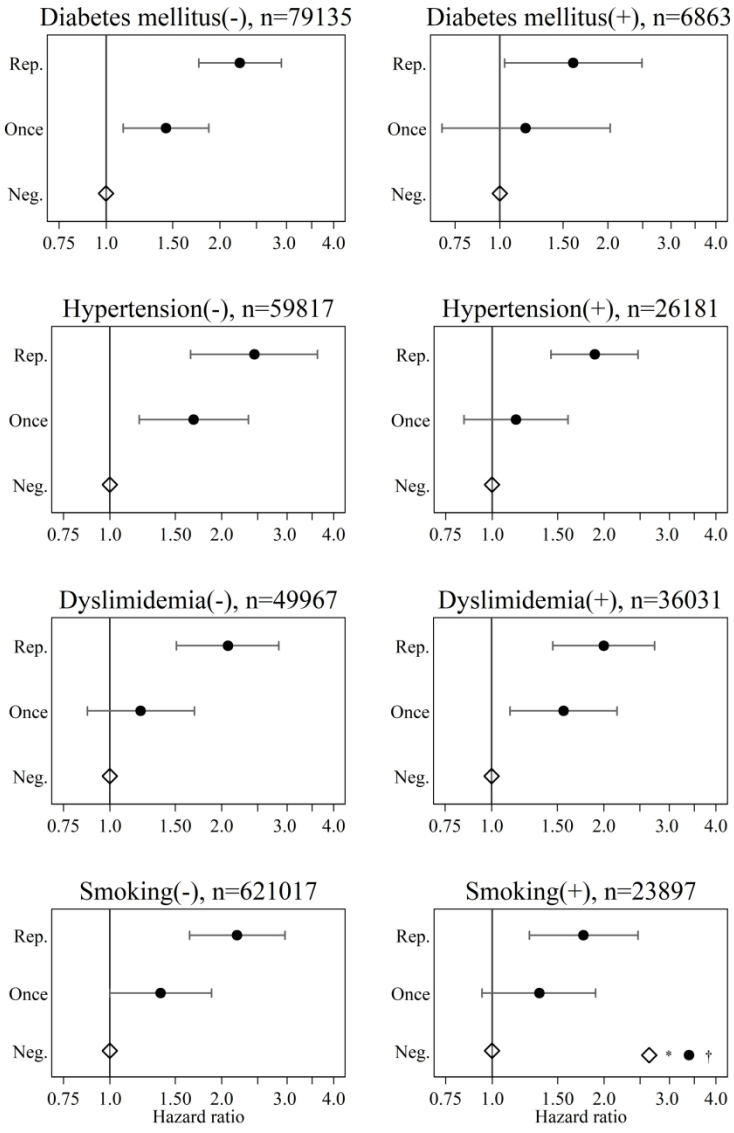
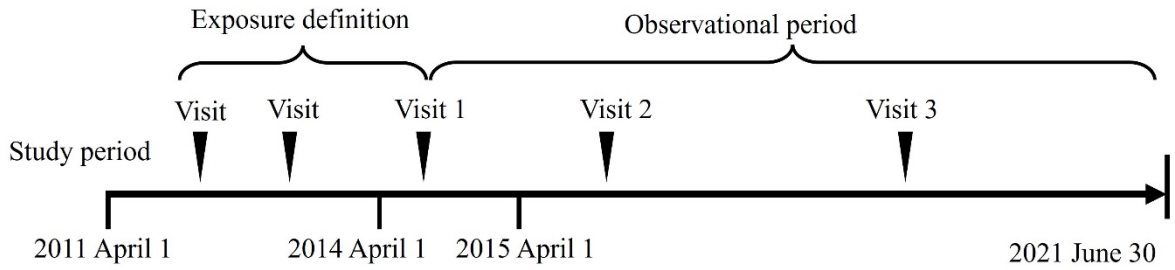


Subgroup analyses by cardiovascular risk factors. *The white squares indicate the hazard ratios in the All-negative group (reference). †The black dots with bars indicate the hazard ratios and 95% confidence intervals in the Once-positive and Repetitively-positive groups. Abbreviations: Neg., All-negative group: Once, Once-positive group: Rep., Repetitively-positive group.

203x304mm (300 x 300 DPI)

Figure S1. Study time course



Visit 1 represents the first visit for health screening after April 1, 2014.

Table S1. Demographic and clinical characteristics of the included and excluded participants

Demographic and clinical characteristics		Included participants	Excluded 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^2		23.7±3.6	23.6±3.7
Estimated glomerular filtration rate, mL/min/1.73 m^2		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%)

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

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^2		23.8±3.7	23.6±3.5
Estimated glomerular filtration rate, mL/min/1.73 m^2		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%)

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

Table S3. Hazard ratios for MACE among patients without major risk factors (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)

*Adjusted for age, sex, and eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, smoking, and alcohol intake.

Table S4. Number of participants with missing variables

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%

Table S5. Demographic and clinical characteristics of participants who had missing data

			N
Age, year		51.2±7.9	2,634
Female, no. (%)		406 (15.4%)	2,634
Body mass index, kg/m^2		24.2±3.6	2,632
Estimated glomerular filtration rate, mL/min/1.73 m^2		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

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

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)

*Adjusted for age, sex, and eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, smoking, and alcohol intake.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	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		we conducted a cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found		Repetitive proteinuria is associated with a greater risk
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported		the difference in CVD risk between single and multiple proteinuria was unclear
Objectives	3	State specific objectives, including any prespecified hypotheses		important knowledge gap in considering effective renal function screening in CVD
Methods				
Study design	4	Present key elements of study design early in the paper	5	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	5	Methods setting and participants section
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5	Setting and participants section
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed		Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7	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	7	Main exposure and covariates section
Bias	9	Describe any efforts to address potential sources of bias	8	Secondary analysis section
Study size	10	Explain how the study size was arrived at	9	Results first paragraph

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8	Main exposure and covariates section
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8	Statistical Analysis section
		(*b*) Describe any methods used to examine subgroups and interactions	8	Secondary Analysis section
		(*c*) Explain how missing data were addressed	8	Secondary analysis section
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	8	Statistical analyses section
		(*e*) Describe any sensitivity analyses	8	Secondary analysis section
Results				
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	8	Result first and second paragraphs
		(b) Give reasons for non-participation at each stage	8	Result first paragraphs
		(c) Consider use of a flow diagram	8	Figure2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	8	Supplemental tableS3
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8	Results second paragraph
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8	Results second paragraph
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*	*Not applicable*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*	*Not applicable*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11	Results second paragraph and Table 2
		(*b*) Report category boundaries when continuous variables were categorized	*NA*	*Not applicable*
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	*NA*	*Not applicable*

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12	Result fourth and fifth paragraph, Table 3
Discussion				
Key results	18	Summarise key results with reference to study objectives	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		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		Discussion fifth paragraph
Generalisability	21	Discuss the generalisability (external validity) of the study results		Discussion fifth paragraph
Other information				
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		Source of finding section

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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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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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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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 ≥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 person-years (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,

cerebrovascular disease, heart failure, and peripheral vascular disease.

Conclusions: Proteinuria is often repeatedly detected during annual renal screening in the general population. Repetitive proteinuria is a risk factor for major cardiovascular events.

Strengths and limitations of this study

- The study had a large number of participants and a long observation period, enabling evaluation of the risk of cardiovascular disease in the general population.
- Evaluation of urinary protein, as an indicator of exposure, could be conducted annually in the general population.
- By using health check-up and insurance data, we accurately tracked the incidence of cardiovascular disease outcomes.
- The semi-quantitative evaluation of urinary protein was a limitation of this study.
- Due to the retrospective study design, the potential impact of residual confounding factors such as the duration of diabetes and the presence of glomerulonephritis cannot be ruled out.

Keywords

proteinuria, cardiovascular diseases, kidney disease, risk

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-to-older 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.

Until recently, therapeutic interventions for proteinuria were mainly limited to renin-angiotensin-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, and this intervention was added to renin-

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.

MATERIALS AND METHODS

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 ≥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); 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 g/day, ≥20 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: I70) [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 ≥2+ and evaluated the association with MACE by excluding the effect of proteinuria ≥2+ on MACE, as participants with repetitive proteinuria were more likely to have proteinuria ≥2+. The redefined categories were as follows: at least one positive result of ≥2+ (2+ positive group), at least two positive results of 1+ with no results ≥2+ (repetitive 1+ positive group), one positive result of 1+ (once 1+ positive group), and all 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 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 ≥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

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).

198 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, years	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^2	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^2	78.4±13.9	78.6±13.3	78.7±14.3	74.8±19.0	<0.001
Baseline proteinuria,* no. (%)					
-	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

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

Abbreviation: LDL, low-density lipoprotein.

*Baseline proteinuria represents urine test results at the beginning of the observation period, which was defined as visit 1

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.

†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.

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

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 person-years for AMI, cerebrovascular disease, HF, and peripheral vascular disease, 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% 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 vascular disease, respectively (Table 3).

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

Secondary outcomes	All-negative group	Once-positive group	Repetitively-positive group
Acute myocardial infarction	Reference	1.s21 (0.78–1.85)	1.85 (1.27–2.70)
Cerebrovascular disease	Reference	1.28 (0.93–1.77)	1.94 (1.44–2.61)
Heart failure	Reference	1.50 (1.05–2.14)	1.88 (1.33–2.68)
Peripheral vascular disease	Reference	1.46 (1.03–2.08)	1.78 (1.26–2.51)

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

*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.

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

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

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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COMPETING INTERESTS

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

AUTHOR CONTRIBUTIONS

Tsuyoshi Ohnishi contributed to the study design, interpretation of the data, and drafting of the manuscript. Yuichiro Mori contributed critically to the revision of the manuscript for important intellectual content. Shingo Fukuma contributed to the study design, data analysis, data interpretation, and critical revision of the manuscript for important intellectual content.

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DATA AVAILABILITY STATEMENT

The data underlying this article are not shared due to the privacy policy of data providers.

SUPPLEMENTAL MATERIAL

Figure S1

Tables S1–S6

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FIGURE LEGENDS

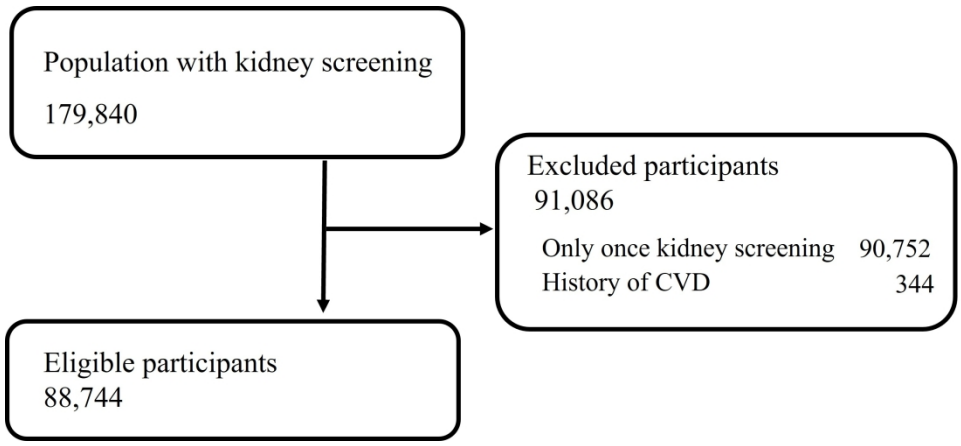
Figure 1. Inclusion and exclusion processes

Figure 2. Subgroup analyses by cardiovascular risk factors

*The white squares indicate the hazard ratios in the All-negative group (reference).

†The black dots with bars indicate the hazard ratios and 95% confidence intervals in the Once-positive and Repetitively-positive groups.

Abbreviations: Neg., All-negative group: Once, Once-positive group: Rep., Repetitively-positive group.



Inclusion and exclusion processes

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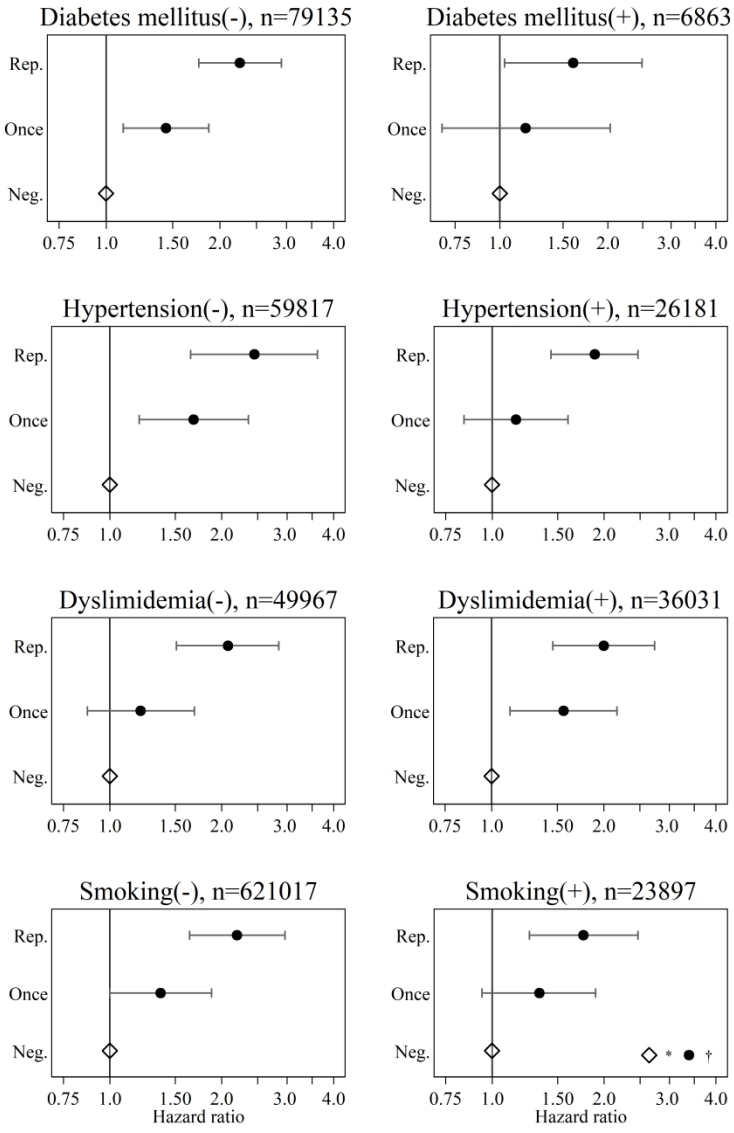

Subgroup analyses by cardiovascular risk factors. *The white squares indicate the hazard ratios in the All-negative group (reference). †The black dots with bars indicate the hazard ratios and 95% confidence intervals in the Once-positive and Repetitively-positive groups. Abbreviations: Neg., All-negative group: Once, Once-positive group: Rep., Repetitively-positive group.

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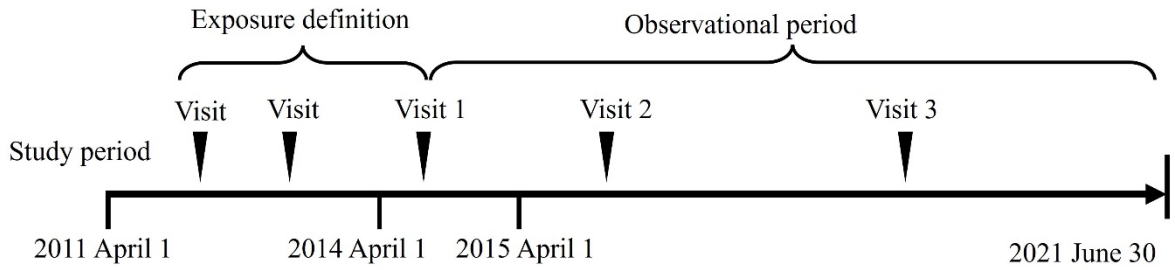
Figure S1. Study time course



Visit 1 represents the first visit for health screening after April 1, 2014.

Table S1. Demographic and clinical characteristics of the included and excluded participants

Demographic and clinical characteristics		Included participants	Excluded 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^2		23.7±3.6	23.6±3.7
Estimated glomerular filtration rate, mL/min/1.73 m^2		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%)

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

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^2		23.8±3.7	23.6±3.5
Estimated glomerular filtration rate, mL/min/1.73 m^2		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%)

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

Table S3. Hazard ratios for MACE among patients without major risk factors (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)

*Adjusted for age, sex, and eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, smoking, and alcohol intake.

Table S4. Number of participants with missing variables

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%

Table S5. Demographic and clinical characteristics of participants who had missing data

			N
Age, year		51.2±7.9	2,634
Female, no. (%)		406 (15.4%)	2,634
Body mass index, kg/m^2		24.2±3.6	2,632
Estimated glomerular filtration rate, mL/min/1.73 m^2		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

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

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)

*Adjusted for age, sex, and eGFR, HbA1c, BMI, SBP, LDL cholesterol, antihypertensive drugs, antidiabetic drugs, antihyperlipidemic drugs, history of myocardial infarction, and history of stroke, smoking, and alcohol intake.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	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		we conducted a cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found		Repetitive proteinuria is associated with a greater risk
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported		the difference in CVD risk between single and multiple proteinuria was unclear
Objectives	3	State specific objectives, including any prespecified hypotheses		important knowledge gap in considering effective renal function screening in CVD
Methods				
Study design	4	Present key elements of study design early in the paper	5	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	5	Methods setting and participants section
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5	Setting and participants section
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed		Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7	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	7	Main exposure and covariates section
Bias	9	Describe any efforts to address potential sources of bias	8	Secondary analysis section
Study size	10	Explain how the study size was arrived at	9	Results first paragraph

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8	Main exposure and covariates section
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8	Statistical Analysis section
		(*b*) Describe any methods used to examine subgroups and interactions	8	Secondary Analysis section
		(*c*) Explain how missing data were addressed	8	Secondary analysis section
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	8	Statistical analyses section
		(*e*) Describe any sensitivity analyses	8	Secondary analysis section
Results				
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	8	Result first and second paragraphs
		(b) Give reasons for non-participation at each stage	8	Result first paragraphs
		(c) Consider use of a flow diagram	8	Figure2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	8	Supplemental tableS3
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8	Results second paragraph
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8	Results second paragraph
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*	*Not applicable*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*	*Not applicable*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11	Results second paragraph and Table 2
		(*b*) Report category boundaries when continuous variables were categorized	*NA*	*Not applicable*
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	*NA*	*Not applicable*

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12	Result fourth and fifth paragraph, Table 3
Discussion				
Key results	18	Summarise key results with reference to study objectives	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		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		Discussion fifth paragraph
Generalisability	21	Discuss the generalisability (external validity) of the study results		Discussion fifth paragraph
Other information				
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		Source of finding section

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.