nitrogen metabolism, and is produced by free ammonia and aspartic acid. Citrulline is normally taken up by the kidneys and converted to urea via arginine. Chuang et al. found significant accumulation of urea cycle intermediates in the patients with end-stage renal disease [21]. Because the kidneys are important in conversion of citrulline to arginine, the increase in the serum level of citrulline in DN patients could be attributed to degradation of this function.

SDMA and asymmetric dimethylarginine (ADMA), which is a structural isomer of SDMA, are formed by the enzymatic methylation of arginine residues within proteins. These metabolites have been identified as biomarkers for chronic kidney disease [22]. ADMA is metabolized by dimethylarginine dimethylaminohydrolase (EC 3.5.3.18) into citrulline and dimethylamine in the kidneys, whereas SDMA is excreted directly into the urine without further modification [23]. In this study, ADMA was under the detection limit, but SDMA was positively correlated with a decrease in function of kidney. Therefore, SDMA is a more sensitive marker than ADMA of various renal diseases, including DN.

Tryptophan is metabolized to kynurenine and further metabolized to acetyl-CoA and NAD in the tryptophan-kynurenine pathway. The rate limiting enzymes of this pathway are indoleamine 2,3-dioxygenase (EC 1.13.11.52) in the kidney and tryptophan 2,3-dioxygenase (EC 1.13.11.11) in the liver. Both these enzymes metabolize tryptophan to *N*-formylkynurenine, and *N*-formylkynurenine is subsequently catabolized to kynurenine. Saito et al. showed the peripheral kynurenine pathway accelerates in renal insufficient rats, and the reaction rate was positively correlated with the severity of the case [24]. They also found increased serum kynurenine concentrations reflected increased tryptophan 2,3-dioxygenase and decreased kynureninase (EC 3.7.1.3) activity in the liver [24]. Integration of profiling of these enzyme activities and metabolites will increase understanding of these mechanisms.

We detected a significant increase in γ-butyrobetaine in DN patients ($p<0.0001$). Toyohara et al. showed a negative correlation between γ-butyrobetaine and eGFR in plasma from the patients with chronic kidney disease [25]. Because γ-butyrobetaine is converted to L-carnitine by γ-butyrobetaine dioxygenase (EC 1.14.11.1), it is assumed the increased γ-butyrobetaine arises from inhibition of this enzyme in the kidney.

The levels of azelaic acid ($p<0.0001$) and galactaric acid ($p<0.0001$) were significantly lower in the DN groups than the non-DN group. These metabolites also showed high negative correlations with UACR (azelaic acid, $r=-0.5210$, $p<0.0001$; galactaric acid, $r=-0.4596$, $p<0.0001$) and positive correlations with eGFR (azelaic acid, $r=0.3739$, $p=0.0007$; galactaric acid, $r=0.4152$, $p=0.0002$). Azelaic acid is a saturated C9 dicarboxylic acid derived from oxidation of fatty acids and inhibits the generation of reactive oxygen species on neutrophils [26]. Galactaric acid, is a natural product found in various fruits, and acts as a growth substrate for many organisms, including *Escherichia coli* [27]. However, biological mechanisms of decreased serum azelaic acid and galactaric acid after onset DN need to be clarified.

In this study, the obtained 19 metabolites showed relatively high separation abilities (AUC values of receiver operating characteristic curves 0.643–0.765, Table 3). To increase the separation ability, we then applied a MLR model to this dataset. The developed MLR model included five metabolites, γ-butyrobetaine, SDMA, azelaic acid, MID 114, and MID 127. This model had a higher AUC value for diagnosis of DN (0.927, $p<0.0001$) than single markers, and shows the use of multiple markers is advantageous (Fig. 2a). However, this model contained two unidentified metabolites. The model using only identified metabolites was even simpler and more versatile for actual diagnosis because it could be used with quantification by another technique, such as LC, LC-MS, or an enzymatic method. Thus, we developed another MLR model using only the identified metabolites, aspartic acid, SDMA, azelaic acid and galactaric acid (Fig. 2b). This model showed high separation ability (AUC value 0.844, $p<0.0001$), and could also be used to diagnose DN. However, there are several limitations to be acknowledged for this study. For example, the developed model should be further validated using larger and independent new datasets. In addition, although we evaluated the generalization ability of the developed model using cross-validation, the specificity of the model was not assessed. Especially, the specificity for DN using data obtained from study of other kidney diseases (e.g., kidney cancer) should be addressed in future study.

In conclusion, we applied CE-MS-based metabolome profiling to serum samples from diabetic patients with or without existing DN. Biomarker candidates for the early diagnosis of DN were obtained. Although a further validation study is needed, this technique has potential as a tool for biomarker discovery studies.

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Predictive Effects of Urinary Liver-Type Fatty Acid–Binding Protein for Deteriorating Renal Function and Incidence of Cardiovascular Disease in Type 2 Diabetic Patients Without Advanced Nephropathy.

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OBJECTIVE—To improve prognosis, it is important to predict the incidence of renal failure and cardiovascular disease in type 2 diabetic patients before the progression to advanced nephropathy. We investigated the predictive effects of urinary liver-type fatty acid–binding protein (L-FABP), which is associated with renal tubulointerstitial damage, in renal and cardiovascular prognosis.

RESEARCH DESIGN AND METHODS—Japanese type 2 diabetic patients (n = 618) with serum creatinine ≤1.0 mg/dL and without overt proteinuria were enrolled between 1996 and 2000, and followed up until 2011. Baseline urinary L-FABP was measured with an enzyme-linked immunosorbent assay. The primary end points were renal and cardiovascular composites (hemodialysis, myocardial infarction, angina pectoris, stroke, cerebral hemorrhage, and peripheral vascular disease). The secondary renal outcomes were the incidence of a 50% decline in estimated glomerular filtration rate (eGFR), progression to an eGFR <30 mL/min/1.73 m^2, and the annual decline rate in eGFR.

RESULTS—During a 12-year median follow-up, 103 primary end points occurred. The incidence rate of the primary end point increased in a stepwise manner with increases in urinary L-FABP. In Cox proportional hazards analysis, the adjusted hazard ratio in patients with the highest tertile of urinary L-FBAP was 1.93 (95% CI 1.13–3.29). This relationship was observed even when analyzed separately in normoalbuminuria and microalbuminuria. Patients with the highest tertile of urinary L-FABP also demonstrated a higher incidence of the secondary renal outcomes.

CONCLUSIONS—Our results indicate that urinary L-FABP may be a predictive marker for renal and cardiovascular prognosis in type 2 diabetic patients without advanced nephropathy.

Patients with type 2 diabetes are at a high risk for the progression to end-stage renal disease (ESRD) and incidence of cardiovascular disease (CVD), both of which are life-threatening complications (1). To improve prognosis in diabetic patients, it is clinically important to identify patients at high risk for these disorders as early as possible and initiate disease management in a timely and appropriate manner.

ESRD and CVD share a number of clinical features and risk factors that are important therapeutic targets. Microalbuminuria is well known to be a common risk factor of ESRD and CVD, and a reduction of urinary albumin excretion (UAE) via any intervention results in a reduced future incidence of these disorders (2,3). However, many patients still develop ESRD and CVD despite improvements in their outcome resulting from recent aggressive multifactorial management (4–6). Thus, we need to explore new predictive markers for these disorders that are independent of UAE.

Renal dysfunction, also referred to as chronic kidney disease (CKD), is also an important predictive factor for ESRD and CVD that is independent of increases in UAE (7,8). There is a growing body of evidence suggesting that tubulointerstitial damage, as well as glomerular damage, contributes to a decline in renal function (9). Thus, measuring factors that relate to the risk of renal tubulointerstitial damage may be potentially useful for identifying patients at higher risk for ESRD and CVD.

Liver-type fatty acid–binding protein (L-FABP), an intracellular carrier protein of free fatty acids, is expressed in the liver and kidney. In the kidney, the expression of L-FABP is predominantly located in the proximal tubules. The high levels of urinary L-FABP were previously suggested to be associated with renal tubulointerstitial damage because excessive reabsorption of free fatty acids into the proximal tubules induces tubulointerstitial damage (10–12). Based on these findings, we conducted a long-term observational study to investigate whether urinary levels of L-FABP were predictive for the progression

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of renal dysfunction and incidence of CVD in patients with type 2 diabetes without advanced nephropathy.

RESEARCH DESIGN AND METHODS

Subject recruitment

Japanese patients with type 2 diabetes were recruited from participants that were registered in the Shiga Prospective Observational Follow-up Study between 1996 and 2000 (13). Patients with cancer, recent occurrences of CVD within the past year, infectious disease, collagen disease, and nondiabetic kidney disease, as confirmed by a renal biopsy, were excluded from the study. After obtaining written informed consent, each individual provided a 24-h urine sample and fasting blood sample at baseline. The serum and urine samples were kept at −80°C, if they were not analyzed immediately. In this study, patients with normoalbuminuria/microalbuminuria and serum creatinine (Cr) ≤1.0 mg/dL were eligible. Based on the UAE rate (UAER) at baseline, patients were classified as having normoalbuminuria (UAER <20 μg/min), microalbuminuria (20≤ UAER <200 μg/min), or overt proteinuria (UAER ≥200 μg/min). Serum concentrations of Cr were measured via an enzymatic method. Finally, 618 patients with normoalbuminuria (n = 422) and microalbuminuria (n = 196) were enrolled and followed up until the end of 2011 or the first occurrence of any renal and cardiovascular composite end points. The participants annually underwent standardized clinical examinations and biochemical tests during the follow-up period. HbA_{1c} levels were presented as National Glycohemoglobin Standardization Program values, according to the recommendations of the Japanese Diabetes Society (14). The study protocol and informed consent procedure were approved by the Ethics Committee of Shiga University of Medical Science.

Measurement of urinary L-FABP

Urinary concentrations of L-FABP were measured using a two-step sandwich enzyme-linked immunosorbent assay (15), and all stored samples obtained at baseline were simultaneously measured in 2002. In this study, the baseline levels of urinary L-FABP in each individual were obtained from one urine sample, as described above. The sensitivity of this assay was >3.0 μg/L. Both of the intra- and interassay coefficients of variation were <10%, respectively. Urinary concentrations of Cr were also measured via an enzymatic method. Urinary excretion levels of L-FABP were expressed as micrograms per gram of Cr.

Follow-up evaluation

The primary end point was the first occurrence of any of the renal and cardiovascular composites, which were as follows: initiation of chronic hemodialysis and the occurrence of myocardial infarction, angina pectoris, stroke, cerebral hemorrhage, peripheral vascular disease (PAD), and death from cardiovascular causes. Myocardial infarction was defined as a clinical presentation characterized by typical symptoms, electrocardiographic changes associated with an elevation of cardiac biomarkers, and angiographic evidence of coronary thrombosis. Angina pectoris was defined as the presence of responsible lesions detected by imaging studies with a history of typical chest pain or electrocardiographic changes and invasive cardiovascular interventions. Stroke, including ischemic stroke and cerebral hemorrhage, was defined as a persistent focal neurologic symptom in which the onset was sudden and was not due to trauma or a tumor and where the responsible lesion was detected by imaging studies. PAD was defined as revascularization with typical symptoms such as cold feet or intermittent claudication. At the annual physical examination of this cohort, we directly examined patients and checked their medical records to identify the onset of primary end points. In a fatal case, the medical record was reviewed by physicians to identify the cause of death. If the cause of death was unclear, it was not counted as a death from cardiovascular cause.

In evaluating the secondary outcomes, we separately assessed CVD events and renal secondary outcomes. In regards to secondary renal outcomes, we assessed two categorical outcomes: a 50% decline in the estimated glomerular filtration rate (eGFR) from baseline and the progression to stage 4 CKD (eGFR <30 mL/min/1.73 m^2) and one outcome as a continuous variable, the annual rate of decline in eGFR over the study period. eGFR was calculated using the simplified prediction equation proposed by the Japanese Society of Nephrology (16): eGFR (mL/min/1.73 m^2) = 194 × [age (years)]$^{-0.287}$ × [serum Cr (mg/dL)]$^{-1.094}$ × 0.739 (for female). At baseline, all participants had an eGFR >60 mL/min/1.73 m^2. In the analysis of the annual rate of decline in eGFR, only patients that were observed over 3 years were used in the estimation of the rate of decline in eGFR. The annual rate of decline in eGFR over the course of the study was determined from the slope of each individual from the linear regression analysis and expressed in mL/min/1.73 m^2/year.

Statistical analysis

Data are expressed as mean ± SD or median (IQR), where appropriate. Patients were divided into tertiles according to the urinary levels of L-FABP at baseline. Statistical significance of the differences among the three subgroups was determined via a χ^2 test for categorical variables, and an ANOVA followed by the Tukey-Kramer test for normally distributed variables or the Kruskal-Wallis test for nonnormally distributed continuous variables. The incidence rate per 1,000 person-years for each outcome was calculated. The cumulative incidence was estimated by using the Kaplan-Meier method and compared with the log-rank test. The follow-up time was censored if any primary end point occurred or if the patient was unavailable for follow-up. The adjusted hazard ratio (HR) for each outcome was evaluated by using a Cox proportional hazards regression model. In this analysis, the known cardiovascular risk factors were age, sex, BMI, HbA_{1c}, total cholesterol, triglycerides, HDL cholesterol, hypertension, use of renin-angiotensin system (RAS) inhibitors, systolic and diastolic blood pressure, past history of CVD, stage of nephropathy (or log UAER for log urinary L-FABP), and eGFR at baseline. The difference of the annual decline rate in eGFR after controlling for the effect of systolic BP and log AER was assessed with the ANCOVA model. All analyses were performed with the SPSS software package (version 11; SPSS Inc., Chicago, IL). A two-sided P value <0.05 was considered statistically significant.

RESULTS—The baseline characteristics of the 618 patients and three subgroups stratified by urinary levels of L-FABP at baseline are presented in Table 1. Age, duration of diabetes, HbA_{1c}, total cholesterol, systolic BP, hypertension, use of RAS inhibitors, urinary AER, microalbuminuria, urinary β_2-microglobulin, and past history of CVD were significantly

Table 1—*Baseline clinical characteristics of all patients with type 2 diabetes and the three subgroups stratified according to the levels of urinary L-FABP*

Variable	All	Urinary L-FABP (μg/g Cr) ≤5.0	5.0–9.5	>9.5	*P* value[a]
n	618	206	206	206	
Male (%)	54.9	49.5	58.3	56.8	NS
Age (years)	59 ± 10	58 ± 10	58 ± 10	62 ± 10	<0.01
BMI (kg/m^2)	23.4 ± 3.3	23.5 ± 3.3	23.4 ± 3.4	23.4 ± 3.3	NS
Duration (years)	11 ± 8	10 ± 7	10 ± 8	13 ± 9	<0.01
Diet/OHA/insulin (%)	25/52/23	31/52/17	26/56/18	17/50/33	<0.01
HbA_{1c} (%)	7.5 ± 1.1	7.5 ± 1.1	7.4 ± 1.0	7.7 ± 1.2	<0.01
Total cholesterol (mg/dL)	213 ± 36	220 ± 34	209 ± 34	212 ± 38	<0.01
HDL cholesterol (mg/dL)	56 (46–66)	57 (47–67)	54 (46–67)	55 (47–64)	NS
Triglycerides (mg/dL)	98 (71–143)	98 (71–148)	96 (69–141)	98 (63–143)	NS
Systolic BP (mmHg)	129 ± 14	127 ± 14	132 ± 14	134 ± 13	<0.01
Diastolic BP (mmHg)	76 ± 10	76 ± 9	77 ± 9	76 ± 11	NS
Hypertension (%)	46.9	40.7	45.1	54.9	<0.05
Using RAS inhibitors (%)	14.2	11.1	10.2	19.9	<0.05
Past history of CVD (%)	10.0	8.2	7.3	14.6	<0.05
Urinary AER (μg/min)	11 (7–27)	8 (5–15)	12 (7–28)	16 (9–43)	<0.01
Microalbuminuria (%)	31.7	18.9	32.5	43.7	<0.01
eGFR (mL/min/1.73 m^2)	88 ± 18	87 ± 18	89 ± 17	87 ± 19	NS
Urinary β_2-microglobulin (μg/g Cr)	120 (81–206)	93 (69–136)	122 (82–183)	175 (106–369)	<0.01
Urinary L-FABP (μg/g Cr)	7.2 (4.2–11.5)	3.4 (2.3–4.3)	7.2 (6.0–8.4)	14.2 (11.4–20.6)	<0.01

Data are expressed as mean ± SD for normally distributed continuous variables or median (IQR) for skewed continuous variables. AER, albumin excretion rate; OHA, oral hypoglycemic agent. [a]Differences between the three subgroups were compared with a χ^2 test for categorical variables and ANOVA for continuous variables.

different between the three subgroups. Additionally, urinary levels of L-FABP in patients with microalbuminuria were higher than in those with normoalbuminuria (9.1 μg/g Cr [IQR 5.9–15.8 μg/g Cr] vs. 6.1 μg/g Cr [3.7–9.9 μg/g Cr]; $P < 0.01$, Mann-Whitney *U* test).

Incidence rates of the primary end point

During a 12-year (IQR 6–15 years) median follow-up, the primary end points occurred in 103 patients (i.e., 7 patients presented with chronic hemodialysis, 25 with myocardial infarction, 35 with angina pectoris, 24 with stroke, 5 with cerebral hemorrhage, and 7 with PAD). The incidence rate per 1,000 person-years of the primary end point was 16.5 in all participants, and increased in a stepwise fashion with increasing urinary levels of L-FABP (i.e., 9.5 in the lowest tertile of urinary L-FABP, 15.5 in the middle tertile, and 25.4 in the highest tertile) (Table 2). As shown in Fig. 1, the cumulative incidences of the primary end point were significantly different among the three subgroups ($P < 0.0001$, log-rank test). The risk for the primary end point was evaluated by using the Cox proportional hazards model (Table 2). When adjusted for known cardiovascular risk factors, the HR in the highest tertile of urinary L-FABP was 1.93 (95% CI 1.13–3.29). Using log urinary L-FABP as a continuous variable, instead of the tertiles of urinary L-FABP, the HR of log urinary L-FABP for primary end points was 2.16 (95% CI 1.23–3.79) after adjusting for age, sex, log UAER, and eGFR at baseline, and 1.79 (1.06–3.01) after adjusting for known cardiovascular risk factors.

Effects of urinary L-FABP on secondary renal outcomes

The incidence rates per 1,000 person-years for a 50% decline in eGFR from baseline in the three subgroups were 4.8 in the lowest tertile, 6.0 in the middle tertile, and 18.3 in the highest tertile. Also, the incidence rates for the progression to stage 4 CKD (eGFR <30 mL/min/1.73 m^2) were 1.8 in the lowest tertile, 2.4 in the middle tertile, and 11.1 in the highest tertile. The adjusted HRs for these secondary renal outcomes were significantly higher in the highest tertile (Table 2). The annual rate of decline in eGFR (mL/min/1.73 m^2/year) was −1.31 (95% CI −0.46 to −2.33) in the lowest tertile, −1.65 (−1.02 to −2.25) in the middle tertile, and −1.80 (−1.05 to −3.21) in the highest tertile ($P = 0.002$, Kruskal-Wallis test), and there was a significant effect of urinary L-FABP on the annual decline rate in eGFR after controlling for the effect of systolic BP and log AER ($F = 3.54$, $P = 0.03$, ANCOVA). In addition, patients in the highest tertile of urinary L-FABP showed the highest incidence of a 50% decline in eGFR, which was associated with the highest incidence of CVD. The cumulative incidence of CVD was significantly higher in patients with a 50% decrease in eGFR than those without it ($P = 0.034$, log-rank test).

Risk of urinary L-FABP according to the stage of diabetic nephropathy

We finally investigated the incidence rates and HRs for the primary end point in the subgroups stratified according to the levels of urinary L-FABP and the stages of diabetic nephropathy at baseline. As shown in Table 3, the incidence rates and HRs adjusted from known cardiovascular risk factors increased with increasing stages of nephropathy and urinary L-FABP levels. Interestingly, the adjusted HR of the subgroups, categorized according to the highest tertile of urinary L-FABP, was significantly higher even in patients with normoalbuminuria. The

Table 2—*Incidence rates and HRs for primary end point and secondary outcomes of patient subgroups stratified according to the levels of urinary L-FABP*

	n	Incidence rate (1,000 person-years)	Adjusted HR (95% CI)[a]		
			Model 1	Model 2	Model 3
Primary end point (hemodialysis and CVD)					
Lowest tertile	21	9.5	1 (reference)	1 (reference)	1 (reference)
Middle tertile	33	15.5	1.60 (0.93–2.77)	1.51 (0.87–2.64)	1.64 (0.93–2.88)
Highest tertile	49	25.4	2.30 (1.37–3.86)	2.04 (1.20–2.69)	1.93 (1.13–3.29)
Secondary end points					
CVD events					
Lowest tertile	19	8.6	1 (reference)	1 (reference)	1 (reference)
Middle tertile	33	15.5	1.75 (0.99–3.09)	1.65 (0.93–2.92)	1.78 (0.99–3.20)
Highest tertile	44	23.4	2.26 (1.31–3.88)	2.00 (1.15–3.49)	1.76 (1.00–3.12)
50% decline in eGFR					
Lowest tertile	10	4.8	1 (reference)	1 (reference)	1 (reference)
Middle tertile	12	6.0	1.27 (0.55–2.94)	1.09 (0.47–2.54)	1.04 (0.44–2.46)
Highest tertile	32	18.3	3.87 (1.89–7.91)	3.09 (1.48–6.45)	2.43 (1.14–5.16)
Progression to stage 4 CKD[b]					
Lowest tertile	4	1.8	1 (reference)	1 (reference)	1 (reference)
Middle tertile	5	2.4	1.27 (0.34–4.74)	1.19 (0.32–4.47)	1.18 (0.30–4.57)
Highest tertile	21	11.1	5.92 (2.02–17.37)	5.05 (1.68–15.21)	3.53 (1.15–10.88)

[a]Adjusted HRs were calculated via the Cox proportional hazards model. Model 1, adjusted for age and sex; model 2, adjusted for age, sex, stage of nephropathy, and eGFR; model 3, adjusted for age, sex, BMI, HbA_{1c}, total cholesterol, log triglycerides, log HDL cholesterol, hypertension, use of RAS inhibitors, systolic and diastolic blood pressure, past history of CVD, stage of nephropathy, and eGFR. [b]Stage 4 CKD denotes eGFR <30 mL/min/1.73 m^2.

effects of diabetic nephropathy and three categories of urinary L-FABP levels were independent of each other (P = 0.34 for interaction).

CONCLUSIONS—The present long-term observational study on type 2 diabetic patients without advanced nephropathy revealed that higher urinary levels of L-FABP were associated with deteriorating renal function and a higher incidence rate of CVD. These associations were observed in those with normoalbuminuria as well as those with microalbuminuria, when separately analyzed according to the stages of diabetic nephropathy. Thus, these findings suggest that urinary L-FABP can be used as a biomarker for predicting future renal dysfunction and incidence of CVD in type 2 diabetic patients with an early stage of nephropathy, in addition to albuminuria.

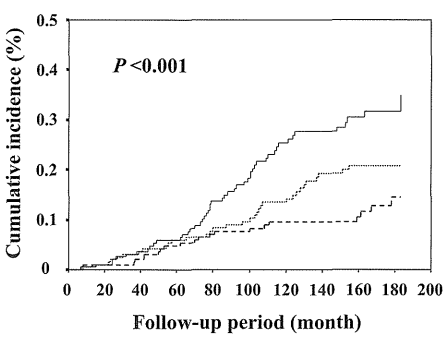

Figure 1—*Kaplan-Meier curves for cumulative incidences of primary end points of the three groups stratified by urinary L-FABP. Solid line, highest tertile group* (n = *206,* ≤*5.0 μg/g Cr); short-dashed line, middle tertile group* (n = *206, 5.0–9.5 μg/g Cr); long-dashed line, lowest tertile group* (n = *206,* >*9.5 μg/g Cr). Differences between groups were compared by a log-rank test.

Renal dysfunction is reported to correlate with the degree of tubulointerstitial damage (9). Although albuminuria per se reflects glomerular damage and subsequently induces renal tubulointerstitial damage, other factors and mechanisms, independent of albuminuria, must be involved in the development of tubulointerstitial damage under diabetic conditions. In fact, a recent study reported on cases where renal function rapidly declined without an increase in UAE (17). Urinary levels of L-FABP have been reported to be associated with the histological severity of renal tubulointerstitial lesions in human (15) and animal studies (18,19). Our study also found that urinary L-FABP correlated with urinary β_2-microglobulin, a marker of renal tubulointerstitial injury. Taken together, these findings suggest that urinary L-FABP may reflect tubulointerstitial damage and, therefore, predict the progression of deteriorating renal function. Furthermore, these results suggest the importance of tubulointerstitial damage in the development of renal dysfunction under diabetic conditions.

In the current study, we focused on the predictive effects of urinary L-FABP for deteriorating renal function and the onset of CVD in type 2 diabetic patients with early stages of nephropathy. Previously, there have been several clinical studies investigating the association between urinary L-FABP levels and the progression of diabetic nephropathy that mainly focused on the progression of nephropathy based on UAE. In a 4-year prospective cohort study on 54 patients with type 2 diabetes, Kamijo-Ikemori et al. (20) reported that higher urinary L-FABP levels were associated with the progression of eGFR to <60 mL/min/1.73 m^2. Additionally, Nielsen et al. (21) reported that higher urinary L-FABP levels predicted all-cause mortality in 165 patients with type 1 diabetes and normoalbuminuria, independent of UAE and other established risk factors. Our findings strengthen these previous results and provide further evidence that urinary L-FABP is a predictive biomarker for renal dysfunction and the onset of CVD in diabetic patients.

However, Nielsen et al. (22) recently reported that urinary L-FABP levels are not related to a rapid decline in GFR

Table 3—*Incidence rates and adjusted HRs for primary end points in patient subgroups stratified according to the levels of urinary L-FABP and stages of diabetic nephropathy*

	Urinary L-FABP		
	Lowest tertile	Middle tertile	Highest tertile
Incidence rate (1,000 person-years)			
Normoalbuminuria	7.8	10.9	21.7
Microalbuminuria	17.8	25.7	31.0
Adjusted HR (95% CI)[a]			
Normoalbuminuria	1 (reference)	1.49 (0.72–3.09)	2.26 (1.15–4.45)
Microalbuminuria	1.72 (0.68–4.38)	2.70 (1.26–5.81)	2.18 (1.08–4.40)

[a]The HRs were adjusted for age, sex, BMI, HbA_{1c}, total cholesterol, log triglycerides, log HDL cholesterol, hypertension, use of RAS inhibitors, systolic and diastolic blood pressure, past history of CVD, and eGFR in the Cox proportional hazards model.

in a 3-year intervention study on 63 type 1 diabetic patients with overt proteinuria. Massive albuminuria per se induces tubulointerstitial damage and then leads to renal dysfunction. Therefore, the effects of urinary L-FABP on tubulointerstitial lesions and decline in GFR may disappear with an increase in albuminuria, such as overt proteinuria. Further investigation is needed to clarify this argument.

CKD, even a mild decline in renal function, is well acknowledged as an important risk factor for cardiovascular morbidity and mortality. A number of diabetic patients with renal dysfunction experience an onset of CVD before they initiate chronic hemodialysis. Also, our study demonstrated a higher incidence of CVD in patients who showed a 50% decline in eGFR during the follow-up than those who did not show a 50% decline.

There are some limitations in this study that must be addressed. In general practice, we do not perform renal biopsies in diabetic patients unless the complication of other renal diseases is suspected. Thus, we could not investigate the correlation between the urinary L-FABP levels and renal lesions in this study. Our study was designed as an observational follow-up study, and not an intervention trial. The treatment protocol for patients in this cohort was not controlled, and the influence of potential cofounders during the observation period was not analyzed. Furthermore, the time-dependent changes of urinary L-FABP levels during the follow-up period were not assessed. Urinary L-FABP may be modified by any intervention (23,24). Thus, a further study is required to answer the important question of whether the changes of urinary L-FABP levels are associated with the prognosis in diabetic patients.

In conclusion, the current study indicated that the high levels of L-FABP in urinary excretion were associated with deteriorating renal function and the high incidence of CVD in patients with type 2 diabetes. This association was markedly observed even in patients with normoalbuminuria. Thus, measurements of urinary L-FABP, in addition to albuminuria, may be clinically useful for the early identification of diabetic patients without advanced nephropathy and at a higher risk for renal disease and CVD. In addition, these results suggest the importance of tubulointerstitial damage in the development of renal dysfunction and CVD under diabetic conditions.

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S.A. designed the study protocol, researched data, and wrote the manuscript. M.H. designed the study protocol, contributed to discussion, and reviewed and edited the manuscript. D.K. researched data, contributed to discussion, and reviewed and edited the manuscript. T.S, K.I., and S.K. researched data. A.K., T.U., and H.M. contributed to discussion and reviewed and edited the manuscript. S.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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ORIGINAL ARTICLE

Risk factor profiles based on estimated glomerular filtration rate and dipstick proteinuria among participants of the Specific Health Check and Guidance System in Japan 2008

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Abstract
Background Estimated glomerular filtration rate (eGFR) and albuminuria (proteinuria) are both important determinants of the risk of cardiovascular disease (CVD), end-stage renal disease (ESRD), and mortality. Few studies, however, have examined the risk factor profiles based on eGFR and proteinuria among the general population.
Methods Data of the newly developed nationwide screening program of the Specific Health Check-up and Guidance System (Tokutei-Kensin) initiated in 2008 were used in this study. The aim of this screening, targeting people 40–74 years of age, was to detect those with metabolic syndrome and to offer those services regarding lifestyle modifications that will lead to the reduction of diabetes mellitus (DM) and DM-related ESRD. Individual records of 580,000 participants in 69 cities and towns and 3 union cohorts throughout Japan were anonymously provided and included in the present study.
Results Details of 332,174 participants (57.3% of the total) with both serum creatinine and dipstick urine test data were analyzed. Mean (SD) age was 63.6 (8.3) years and 40.6% were men. The mean (SD) eGFR was 75.0 (16.2) ml/min/1.73 m^2 and 5.4% had proteinuria. The prevalence of chronic kidney disease (CKD) stage 3, 4, and 5 was 14.2%, 0.2%, and 0.07%, respectively. The prevalence of DM, hypertension, and history of stroke and heart disease was correlated with the combination of eGFR and degree of proteinuria.
Conclusion The findings of the present study indicate that CKD and risk factors for CVD are quite common among middle-aged Japanese. CKD classification based on eGFR and proteinuria may be useful for predicting CVD, mortality rate, and ESRD in the Japanese population.

Keywords eGFR · CKD · Screening · Proteinuria · Epidemiology

Introduction

Chronic kidney disease (CKD) is a common condition and is a risk factor for developing cardiovascular disease (CVD) and end-stage renal disease (ESRD) [1]. Both the prevalence and incidence of treated ESRD are very high in Japan [2]. Furthermore, the incidence and prevalence continue to increase, despite several preventive strategies aimed at early detection and treatment of CKD. Japan has a long history of universal screening, a program that might facilitate the early detection of CKD [3]. A higher mean age at the start of dialysis can be interpreted as delaying the progression of CKD, but it may also simply reflect the increase in the elderly population and longevity. Dipstick proteinuria is a strong predictor of developing ESRD in a setting of community-based screening [4]. Delayed visits to the nephrology clinic result in an inevitable initiation of

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dialysis with a short duration of follow-ups [5, 6]. Such 'late referral' negatively impacts survival after dialysis is initiated. Preliminary results of the Japanese Society Dialysis Therapy support the notion that the longer the duration of pre-ESRD treatment, the better the survival. Because CKD remains asymptomatic until the late stages, effective strategies for the early detection and treatment of CKD are necessary.

The increasing prevalence of obesity and diabetes mellitus (DM) has become the leading cause of ESRD. A specific nationwide health check-up and guidance system, called Tokutei-Kenshin, was initiated in April 2008 in Japan (The Ministry of Health, Labour and Welfare; http://www-admin@.mhlw.go.jp). The aim of this project is to detect metabolic syndrome and if confirmed, to provide individual instruction to modify lifestyle and the necessary treatment. The target population comprises Japanese citizens between the ages of 40–74 years. Data on the prevalence of risk factors for developing CKD, ESRD, and CVD are limited to the Japanese population. In the present study, we examined the demographics of participants of the newly developed screening system in Japan. Risk factor profiles were examined according to the new CKD classification based on the combination of estimated glomerular filtration rate (eGFR) and dipstick proteinuria findings [7]. Results of dipstick proteinuria were categorized into three groups: (−) and (±), 1+, and ≥2+. The present study provides the baseline characteristics for the future outcome study as the unique identification number was set by the government.

Methods

Individual records for 580,000 participants in 12 communities or prefectures were anonymously provided and included in this analysis. Among these participants, subjects with data for both serum creatinine and dipstick proteinuria were selected for this study. A test was mandatory for dipstick proteinuria, but not for serum creatinine. Therefore, rates of measurement of serum creatinine differ among cohorts or prefectures. Databases included in this study were from Yamagata, Miyagi, Fukushima, Ibaraki, Tokyo, Kanagawa, Niigata, Osaka, Okayama, Kochi, Fukuoka, Miyazaki and Okinawa, and ethical approval was obtained from the respective institute review boards. Data were sent to a data center called the NPO Japan Clinical Research Support Unit to be verified. Outliers were deleted through winsorization and accounted for 0.01–0.1% of the total. Eligible participants visited a pre-assigned clinic or hospital and responded to a questionnaire regarding past history of stroke, cardiac disease, kidney disease, lifestyle habits such as smoking, alcohol intake, walking, etc., and medications for hypertension, DM, and dyslipidemia. Screening participants are eligible for public support for the standard health checks, such as measurement of height, weight, waist circumference, blood pressure, fasting blood glucose, hemoglobin A1c, triglyceride, serum high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, glutamyl oxaloacetic transaminase, glutamate pyruvate transaminase, gamma-glutamyl transpeptidase, hemoglobin, uric acid, serum creatinine, dipstick urine test for proteinuria, hematuria, and glucosuria. Proteinuria was coded as (−), (±), (1+), (2+), and (3+). Serum creatinine was measured using the enzymatic method. Glomerular filtration rate was calculated using the formula of the Japanese Society of Nephrology [8]. Reference levels for triglyceride, HDL cholesterol, LDL cholesterol, uric acid, fasting blood glucose, and hemoglobin A1c were set at 150, 40, 7, 110 mg/dl, and 6.1%, respectively. Blood pressure was measured in all cohorts using a standard sphygmomanometer. Hypertension was defined as ≥140/90 mmHg or on antihypertensive medication. DM was defined as hemoglobin A1c ≥6.1% or on medication for DM. Obesity was defined as body mass index (BMI) ≥25 kg/m^2.

Statistical analysis

Data were analyzed with SAS/STAT software (version 6.03, SAS Institute, Tokyo, Japan). Student's *t* test and the Chi-squared test were performed to compare the significance of discrete variables. A *P* value of less than 0.05 was considered statistically significant in all analyses.

Results

Demographics of the screened subjects are summarized in Table 1. The prevalence of CKD (i.e., eGFR <60 ml/min/1.73 m^2) was as high as 14.2%. Compared to national statistics, smoking rates were lower in both men and women than in the general population. Those with low eGFR comprised 14.2% and proteinuria was distributed as follows: negative and ± 94.55%, 1+ 3.75%, and ≥2+ 1.7%.

The prevalence of obesity, DM, and hypertension is summarized based on the results of eGFR and proteinuria in Table 2. The prevalence of obesity, DM, and hypertension increased in relation to the degree of proteinuria in each eGFR group. Higher levels of proteinuria together with lower levels of eGFR were associated with an increased prevalence of hypertension (Fig. 1).

History of stroke, heart disease, and CVD (either stroke or heart disease) is summarized in Table 3. The prevalence of CVD was highest (25.2%) in those with proteinuria of (1+) and an eGFR of 15–29 ml/min/1.73 m^2, and the prevalence was lowest (6.1%) in those negative and ± for

Table 1 Demographics of the screened cohorts. Screening was performed during April 1, 2008 to March 31, 2009

Number of participants	332,174		
Men (%)	134,751 (40.6)		
Age (years)	63.6 (8.3), 40–74		
Body height (cm)	157.2 (8.5)	Men 164.6 (6.3)[#]	Women 152.2 (5.7)
Body weight (kg)	57.6 (10.5)	Men 64.5 (9.5)[#]	Women 52.8 (8.3)
Body mass index (kg/m^2)	23.2 (3.3)	Men 23.8 (3.1)[#]	Women 22.8 (3.5)
Waist circumference (cm)	84.1 (9.2)	Men 85.7 (8.3)[#]	Women 83 (9.5)
Systolic blood pressure (mmHg)	128.9 (17.4)		
Diastolic blood pressure (mmHg)	76.3 (10.7)		
Fasting blood glucose (mg/dl)	98.2 (21.5)		
Hemoglobin A1c (%)	5.3 (0.7)		
Triglyceride (mg/dl)	122.5 (84.0)		
HDL cholesterol (mg/dl)	62.1 (16.3)		
LDL cholesterol (mg/dl)	125.9 (30.6)		
Hemoglobin (g/dl)	13.6 (1.4)		
Serum creatinine (mg/dl)	0.7 (0.2)	Men 0.8 (0.3)[#]	Women 0.6 (0.2)
eGFR (ml/min/1.73 m^2)	75.0 (16.2)		
<15	240 (0.07%)		
15–29	655 (0.20%)		
30–44	4,300 (1.29%)		
45–59	42,975 (12.94%)		
60–89	225,081 (67.76%)		
≥90	58,923 (17.74%)		
Serum uric acid (mg/dl)	5.2 (1.4)	Men 6.0 (1.3)[#]	Women 4.7 (1.1)
Glucosuria[a]	2.30%		
Proteinuria[a]	5.40%		
Hematuria[a]	7.50%		
Past history (%)			
Stroke	3.30		
Cardiac disease	6.00		
Kidney disease	0.70		
Medication (%)			
Anti-hypertensive drugs	28.80		
Lipid lowering drugs	15.80		
Insulin or hypoglycemic drugs	5.20		
Lifestyle (%)			
Smoking	13.50	Men 25.2%[#]	Women 5.5%
Drinking	44.30	Men 65.2%[#]	Women 30.0%

Data are mean (SD)

[a] Positive urine test denote ≥1+ by dipstick

[#] $P < 0.01$ (vs women)

proteinuria and having an eGFR ≥90 ml/min/1.73 m^2. The combination of higher levels of proteinuria and lower levels of eGFR was associated with an increased prevalence of a history of CVD (Fig. 2).

Mean (SD) levels of BMI and smoking rate are summarized in Table 4. Both BMI and smoking rate were higher in men than in women. The smoking rate tended to decrease in the lower eGFR category.

Discussion

The target population of this screening in Japan comprised participants from 40 to 74 years of age, and the expected turnout was approximately 58 million. In the 2008 screening, the actual participation rate remained low, 20–30%, probably because of the lack of preparation for implementing this new system. The total number of participants in the present study was approximately 0.58 million; therefore, our analysis included at least 1% of the target population in Japan.

The results revealed the current health status among the general Japanese population. The proportion of the population comprising elderly people is high in Japan and its rate of increase is currently the highest in the world. The proportion of those with a low GFR (<60 ml/min/1.73 m^2), regardless of proteinuria, increases with aging. Fortunately, the rate of decline of the GFR in the Japanese is relatively low, 0.36 ml/min/1.73 m^2/year [9]. Elderly people are at risk for CVD and death. Effective strategies to establish a health check and guidance system are necessary to better accommodate the future burden of medical and social costs due to the aging population. Based on the findings of the present study, we propose that a cost–benefit analysis be performed on programs designed for the early detection and treatment of CKD, including education regarding lifestyle modification.

CVD is a recently recognized risk factor for CKD and ESRD [7]. The prevalence of CVD in CKD stage 5 is

Table 2 Prevalence of obesity, DM, and hypertension based on the combination of eGFR and proteinuria

eGFR	Proteinuria	Number (%)	Obesity (%)	DM (%)	Hypertension (%)
<15	Minus, ±	101 (0.03)	22.8	10.2	50.5#
	1+	35 (0.01)	28.6	17.6	88.6*
	≥2+	103 (0.03)	31.1	32.0*	92.2*
15–29	Minus, ±	251 (0.08)	29.1	25.2*	81.1*
	1+	119 (0.04)	31.1	22.7*	85.5*
	≥2+	285 (0.09)	40.3*	38.4*	91.9*
30–44	Minus, ±	3,194 (0.96)	35.8*	13.9*	68.2*
	1+	504 (0.15)	43.8*	24.9*	83.3*
	≥2+	579 (0.17)	46.5*	33.9*	85.9*
45–59	Minus, ±	39,265 (11.82)	30.7*	9.3*	52.7*
	1+	2,408 (0.72)	42.2*	19.6*	71.6*
	≥2+	1,326 (0.40)	49.5*	31.1*	80.2*
60–89	Minus, ±	214,768 (64.66)	25.4*	8.4*	43.3*
	1+	7,579 (2.28)	38.5*	19.3*	62.1*
	≥2+	2,703 (0.81)	46.5*	31.1*	72.0*
≥90	Minus, ±	56,495 (17.01)	24.3	10.6	39.8
	1+	1,812 (0.55)	37.9*	26.5*	57.4*
	≥2+	647 (0.19)	46.5*	36.4*	72.0*

Total number of participants was 332,174. Parentheses are the percentage to the total participants in each column. Obesity, BMI ≥25 kg/m^2; DM, HbA1c ≥6.1% or on treatment; hypertension, 140/90 mmHg or on treatment

* $P < 0.0001$, $^{\#}P < 0.05$ (vs. reference value of eGFR ≥90 and proteinuria minus or ±)

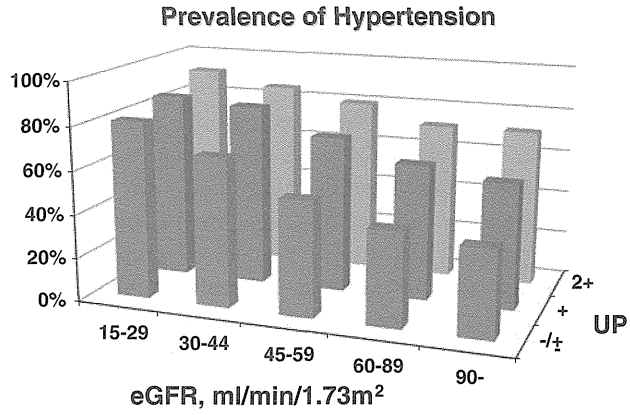

Fig. 1 Prevalence of hypertension by the combination of eGFR and proteinuria. Prevalence of hypertension was significantly ($P < 0.0001$) higher in every column except those with eGFR 15–29 and proteinuria minus or (±) ($P < 0.05$) when compared to the reference value of eGFR ≥90 and proteinuria minus or (±)

Table 3 Prevalence of history of stroke and heart disease based on the combination of eGFR and proteinuria

eGFR	Proteinuria	Stroke	Heart Disease	CVD
<15	Minus, ±	4.0	6.9	10.9*
	1+	5.7	17.1*	22.9
	≥2+	8.7*	12.6	19.4#
15–29	Minus, ±	13.9*	15.9*	25.1*
	1+	15.1*	15.1*	25.2*
	≥2+	11.6*	13.7*	22.5*
30–44	Minus, ±	8.6*	13.1*	19.2*
	1+	9.9*	16.1*	22.4*
	≥2+	10.5*	16.4*	23.7*
45–59	Minus, ±	4.8*	8.5*	12.3*
	1+	6.9*	11.7*	16.2*
	≥2+	8.3*	13.1*	19.2*
60–89	Minus, ±	3.0*	5.6*	8.1*
	1+	4.7*	7.3*	11.1*
	≥2+	5.8*	9.3*	13.8*
≥90	Minus, ±	2.4	4.1	6.1
	1+	3.5†	6.0*	8.8*
	≥2+	4.5†	4.6	8.5†

Total number of participants was 332,174. Cardiovascular disease (CVD) denotes stroke and/or heart disease

* $P < 0.0001$, †$P < 0.02$, #$P < 0.05$ (vs reference value of eGFR ≥90 and proteinuria minus or ±)

approximately 25%; similar to the prevalent dialysis population. Ethnic variations in CVD incidence and subtype are well described in the general population [10, 11]. The stroke mortality rate is high in Japan; however, in the present study, the prevalence of stroke was lower than that of cardiac disease (Table 1). The reasons for this finding are not clear, but many people with stroke are unable to participate in this type of screening program.

Metabolic syndrome is an important risk factor for developing CKD [12], and for DM and hypertension, which are the main causes of ESRD [13]. We previously reported the significance of obesity in the risk for ESRD. Recent societal changes in lifestyle related to motorized transportation and high-calorie intake may have contributed to the

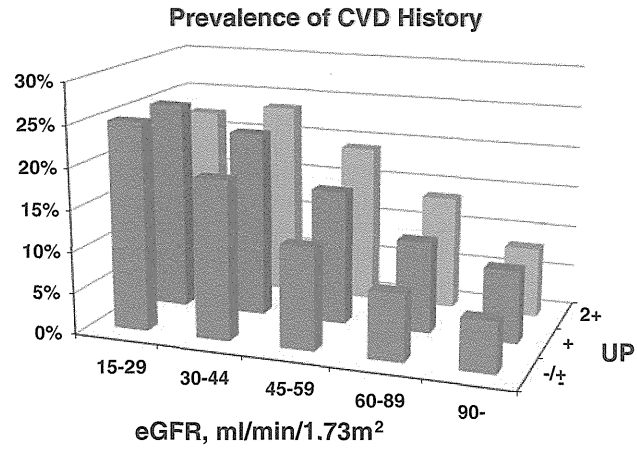

Fig. 2 Prevalence of history of cardiovascular disease (*CVD*) by the combination of eGFR and proteinuria. Prevalence of CVD was significantly ($P < 0.0001$) higher in every column except those with eGFR 15–29; not significant for proteinuria (+), and $P < 0.05$ for proteinuria ≥2+, when compared to the reference value of eGFR ≥90 and proteinuria minus or (±). *P* value was <0.02 for those with eGFR ≥90 and proteinuria ≥2+

Table 4 Mean (SD) levels of body mass index (BMI) and smoking rate in each sex based on the combination of eGFR and proteinuria

eGFR	Proteinuria	Men		Women	
		BMI (kg/m^2)	Smoker (%)	BMI (kg/m^2)	Smoker (%)
<15	Minus, ±	24.1 (2.6)	5.4	22.2 (3.0)	10.9
	1+	24.2 (2.4)	12.5	22.0 (3.4)	5.3
	≥2+	23.3 (2.8)	22.2	24.5 (4.7)	2.5
15–29	Minus, ±	23.6 (3.1)	15.0	23.6 (4.1)	8.0
	1+	24.5 (3.4)	7.0	23.5 (3.8)	6.5
	≥2+	24.2 (3.1)	18.4	25.3 (4.7)	6.0
30–44	Minus, ±	24.3 (2.9)	15.3	23.7 (3.8)	4.4
	1+	24.8 (3.5)	19.5	24.2 (4.5)	6.6
	≥2+	25.2 (3.2)	19.5	24.9 (4.4)	5.9
45–59	Minus, ±	24.1 (2.8)	15.8	23.2 (3.4)	3.9
	1+	24.7 (3.0)	20.6	24.2 (4.2)	5.7
	≥2+	25.2 (3.5)	24.9	25.1 (4.4)	5.7
60–89	Minus, ±	23.7 (3.0)	24.4	22.7 (3.4)	5.1
	1+	24.5 (3.4)	29.4	23.9 (4.3)	6.8
	≥2+	25.1 (3.8)	31.2	24.8 (4.8)	8.1
≥90	Minus, ±	23.4 (3.4)	38.8	22.7 (3.6)	7.2
	1+	24.2 (4.0)	46.5	24.2 (4.5)	8.3
	≥2+	25.0 (4.2)	39.5	25.0 (5.0)	9.4

Total number of participants was 332,174

SD standard deviation

increased prevalence of obesity. Although the prevalence of obesity (BMI ≥30 kg/m^2) is lower in Japan than in the USA [14], complications begin to increase in the Japanese after reaching a BMI of 25 kg/m^2.

Microalbuminuria is suspected when the dipstick test results for proteinuria are (±) and/or 1+ [15]. Routine measurement of microalbuminuria is not feasible for the universal screening of CKD, as the cost is much higher than that of a dipstick urine test for proteinuria. Japan has a long history of universal screening, including dipstick urine testing for both proteinuria and hematuria. A positive proteinuria test result has a strong predictive value for the development of ESRD.

The strengths of the present study are: the number of participants was sufficiently large. It is the first nationwide targeted screening program aimed at determining the prevalence of metabolic syndrome in Japan. People diagnosed with metabolic syndrome are entitled to receive instruction to modify their lifestyles and therefore the risk factors for CKD and CVD can be modified accordingly. The prevalence of metabolic syndrome and obesity, particularly in men, is increasing; therefore, the prevalence of CKD is increasing in Japan [16].The combined eGFR and dipstick proteinuria test results indicate that the prevalence of risk factors for CKD and CVD increasing. Future follow-up studies will provide the predictive value of this CKD stratification on CVD, ESRD, and mortality.

The present study has several limitations. It is a cross-sectional study. Single tests for dipstick proteinuria and serum creatinine might cause misclassification of the true prevalence of CKD. To confirm the existence of CKD, the test should be repeated annually, at least 3 months apart. The current estimation of GFR used in this study is precise (<60 ml/min/1.73 m^2); therefore, the proportion of those with moderately decreased GFR (<45 ml/min/1.73 m^2) seems to be high, 1.56%. We selected patients with data for both serum creatinine and dipstick urine test, which comprised approximately two-thirds of the total participants. A cost–benefit analysis on the best combination of screening tests remains to be performed in Japan. Details of CVD, such as subtype of stroke and heart disease, are not clear. Risk factors may differ among diseases. Information of past medical history, medications, and lifestyle were obtained from a questionnaire, which has not yet been validated. Finally, the elderly population, those aged ≥75 years, was not considered in the present screening. It remains to be determined whether or not risk stratification based on both eGFR and proteinuria is applicable in this age group. CKD also has a role in medical problems commonly seen in elderly people, such as malignancies, pneumonia, sepsis, dementia, and bone fractures.

In conclusion, the risk profiles of CKD and CVD are indicated by the new CKD classification based on eGFR and proteinuria levels in the newly developed screening system used in Japan. Although CKD stratification based on the combined eGFR and proteinuria results seems to be a useful predictor of CVD and mortality in the general

population in Japan, the validity of this finding has yet to be demonstrated in outcome studies, and would be useful for the international comparison of the incidence of ESRD [17].

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Conflict of Interest None.

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ORIGINAL ARTICLE

Cost-effectiveness of chronic kidney disease mass screening test in Japan

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Abstract
Background Chronic kidney disease (CKD) is a significant public health problem. Strategy for its early detection is still controversial. This study aims to assess the cost-effectiveness of population strategy, i.e. mass screening, and Japan's health checkup reform.
Methods Cost-effectiveness analysis was carried out to compare test modalities in the context of reforming Japan's mandatory annual health checkup for adults. A decision tree and Markov model with societal perspective were constructed to compare dipstick test to check proteinuria only, serum creatinine (Cr) assay only, or both.
Results Incremental cost-effectiveness ratios (ICERs) of mass screening compared with do-nothing were calculated as ¥1,139,399/QALY (US $12,660/QALY) for dipstick test only, ¥8,122,492/QALY (US $90,250/QALY) for serum Cr assay only and ¥8,235,431/QALY (US $91,505/QALY) for both. ICERs associated with the reform were calculated as ¥9,325,663/QALY (US $103,618/QALY) for mandating serum Cr assay in addition to the currently used mandatory dipstick test, and ¥9,001,414/QALY (US $100,016/QALY) for mandating serum Cr assay and applying dipstick test at discretion.
Conclusions Taking a threshold to judge cost-effectiveness according to World Health Organization's recommendation, i.e. three times gross domestic product per capita of ¥11.5 million/QALY (US $128 thousand/QALY), a policy that mandates serum Cr assay is cost-effective. The choice of continuing the current policy which mandates dipstick test only is also cost-effective. Our results suggest that a population strategy for CKD detection such as mass screening using dipstick test and/or serum Cr assay can be justified as an efficient use of health care resources in a

On behalf of The Japanese Society of Nephrology Task Force for the Validation of Urine Examination as a Universal Screening.

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population with high prevalence of the disease such as in Japan and Asian countries.

Keywords Chronic kidney disease · Cost-effectiveness · Dipstick test · Mass screening · Proteinuria · Serum creatinine

Introduction

A consensus has been established that chronic kidney disease (CKD) is a worldwide public health problem [1, 2]. The effectiveness of its early detection and treatment to prevent progression to end-stage renal disease (ESRD) and premature death from cardiovascular disease has become widely accepted [3], while the strategy of its screening is still under debate [4]. Whereas high-risk strategies such as routine screening for diabetes patients and as a part of initial evaluation of hypertension patients are pursued in Western countries [5, 6], some argue that population strategies, such as mass screening, could be adopted in Asian countries where CKD prevalence is high [7].

Japan has a long history of mass screening programme for kidney diseases targeting school children and adults since the 1970s. Both urinalysis and measurement of serum creatinine (Cr) level have been mandated to detect glomerulonephritis in annual health checkup provided by workplace and community for adults aged ≥40 years old since 1992 [8]. However, glomerulonephritis was replaced as the leading cause of ESRD by diabetic nephropathy in 1998, and the focus of mass screening policy for adults was shifted to control of lifestyle-related diseases. In 2008, the Japanese government launched a programme, Specific Health Checkup (SHC) and Specific Counselling Guidance, focusing on metabolic syndrome in order to control lifestyle-related diseases, targeting all adults between the ages of 40 and 74 years [9]. This is a combined programme of mass screening followed by health education or referral to physicians. During the process of this development of SHC, different types of screening test for kidney diseases were discussed in the health policy arena [10]. Abandonment of dipstick test to check proteinuria was initially proposed by the Ministry of Health, Labour and Welfare, which was opposed by nephrologists who emphasised the significance of CKD. As a consequence, serum Cr assay was alternatively dropped and dipstick test remained in the list of mandatory test items [11]. However, those found with proteinuria in SHC are not included in the health education programme nor referred to physicians in the following Specific Counselling Guidance that particularly targets metabolic syndrome. At the time, much attention was paid to a report from the USA which suggested the cost-ineffectiveness of mass screening for proteinuria [12], which encouraged the government to abandon dipstick test in their initial proposal.

From the viewpoint of CKD control, the current SHC and Specific Counselling Guidance are not adequate. Therefore, to present evidence regarding CKD screening test for the revision of SHC, which is due in 5 years from its start in 2008, the Japanese Society of Nephrology set up the Task Force for the Validation of Urine Examination as a Universal Screening. Since cost-effectiveness analysis provides crucial information for organising public health programmes such as mass screening, the task force conducted an economic evaluation as a part of their mission. This paper presents the value for money of CKD screening test demonstrated by the task force. The results have implications for CKD screening programmes not only in Japan but also for other populations with high prevalence of CKD such as in Asian countries.

Methods

We conducted cost-effectiveness analysis of CKD screening test in SHC with a decision tree and Markov modelling from societal perspective in Japan. In modelling, we carried out a deliberate literature survey to find the best available evidence from Japan, while reports from overseas were excluded. The PubMed database and Igaku Chuo Zasshi (Japana Centra Revuo Medicina), a Japanese medical literature database, were accessed with combinations of relevant terms such as CKD, health checkup etc. Additionally, we re-analysed our databases and carried out surveys where applicable.

Participant cohort

We assume that uptake of SHC does not change regardless of the choice of the test used for CKD screening, so we model a cohort of participants in SHC. Since the sex and age distribution of participants affects outcomes, we run our economic model by sex and age strata. Probabilities of falling into a sex and age stratum are adopted from a nationwide complete count report of SHC in 2008 [13]. Each value is shown in Table 1, and we estimate outcomes based on the prognosis of participants by initial renal function. We also run our economic model for 25 initial renal function strata defined by the combination of five levels of dipstick test results and five stages of CKD according to estimated glomerular filtration rate (eGFR) derived from serum Cr level. Probabilities of falling into an initial renal function stratum are calculated from the Japan Tokutei-Kenshin CKD Cohort 2008, which is a large cohort for the evaluation of SHC. Each value is shown in Table 1.

Table 1 Model assumptions

			Base-case value	Range tested in sensitivity analysis (%)	Source
Participant cohort					
Probability (%)					
Falling into sex and age stratum	Male	40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74	10.008, 9.280, 8.810, 9.783, 6.460, 5.721, 4.472	±50	[13]
	Female	40–44, 45-49, 50–54, 55–59, 60–64, 65–69, 70–74	6.291, 6.054, 6.137, 7.364, 6.836, 7.143, 5.643		
Falling into initial renal function stratum	−	Stage 1, stage 2, stage 3, stage 4, stage 5	11.660, 46.095, 28.627, 0.224, 0.029	±50	Japan Tokutei-Kenshin CKD Cohort 2008
	±	Stage 1, stage 2, stage 3, stage 4, stage 5	0.866, 3.771, 3.214, 0.056, 0.008		
	1+	Stage 1, stage 2, stage 3, stage 4, stage 5	0.325, 1.548, 1.779, 0.086, 0.013		
	2+	Stage 1, stage 2, stage 3, stage 4, stage 5	0.080, 0.385, 0.705, 0.095, 0.026		
	≥3+	Stage 1, stage 2, stage 3, stage 4, stage 5	0.027, 0.104, 0.204, 0.053, 0.020		
Decision tree					
Probability (%)					
Seeking detailed examination after screened as further examination required			40.0	±50	[15, 16] and expert opinion
Either eGFR <50 ml/min/1.73 m^2 or having comorbidity among stage 3 patients (advanced stage 3)			83.5	±50	Japan Tokutei-Kenshin CKD Cohort 2008
Starting CKD treatment after detailed examination	−	Advanced stage 3, stage 4, stage 5	48.9, 82.2, 96.0	±50	Delphi method survey of expert committee
	±	Advanced stage 3, stage 4, stage 5	51.7, 83.9, 97.1		
	1+	Stage 1, stage 2, early stage 3, advanced stage 3, stage 4, stage 5	25.6, 31.1, 46.7, 71.7, 92.2, 98.0		
	2+	Stage 1, stage 2, early stage 3, advanced stage 3, stage 4, stage 5	62.2, 68.3, 78.9, 93.2, 97.1, 99.8		
	≥3+	Stage 1, stage 2, early stage 3, advanced stage 3, stage 4, stage 5	93.2, 94.3, 97.1, 97.7, 99.9, 99.9		
Markov model					
Probability (%)					
From (1) screened and/or examined to (2) ESRD with no treatment by initial renal function	−	Stage 1, stage 2, stage 3, stage 4, stage 5	0.001, 0.004, 0.016, 0.154, 1.743	±50	Calculated from Okinawa database [18]
	±	Stage 1, stage 2, stage 3, stage 4, stage 5	0.019, 0.020, 0.036, 1.137, 5.628		
	1+	Stage 1, stage 2, stage 3, stage 4, stage 5	0.036, 0.024, 0.303, 3.527, 15.802		
	2+	Stage 1, stage 2, stage 3, stage 4, stage 5	0.080, 0.305, 1.170, 10.939, 31.409		
	≥3+	Stage 1, stage 2, stage 3, stage 4, stage 5	0.347, 0.933, 2.506, 13.824, 69.340		

Table 1 continued

				Base-case value	Range tested in sensitivity analysis (%)	Source
From (2) ESRD to (5) death by sex and age	Male	40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90		0.033, 0.034, 0.035, 0.036, 0.038, 0.039, 0.041, 0.042, 0.044, 0.045, 0.047, 0.048, 0.050, 0.052, 0.054, 0.056, 0.058, 0.060, 0.062, 0.065, 0.068, 0.071, 0.074, 0.078, 0.081, 0.084, 0.088, 0.092, 0.097, 0.101, 0.105, 0.111, 0.117, 0.123, 0.129, 0.135, 0.142, 0.148, 0.155, 0.160, 0.166, 0.176, 0.186, 0.196, 0.202, 0.208, 0.226, 0.229, 0.245, 0.288, 0.257	±50	Calculated from Japanese dialysis patient registry [21]
	Female	40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90		0.029, 0.030, 0.031, 0.032, 0.033, 0.034, 0.035, 0.036, 0.038, 0.039, 0.041, 0.042, 0.043, 0.045, 0.047, 0.049, 0.050, 0.052, 0.055, 0.057, 0.059, 0.062, 0.065, 0.068, 0.070, 0.074, 0.078, 0.080, 0.085, 0.089, 0.093, 0.097, 0.101, 0.105, 0.110, 0.115, 0.122, 0.127, 0.134, 0.138, 0.145, 0.151, 0.159, 0.162, 0.173, 0.185, 0.188, 0.198, 0.205, 0.219, 0.236		
From (1) screened and/or examined to (3) heart attack with no treatment by initial dipstick test result, sex and age	<1+	Male	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.005, 0.041, 0.076, 0.132, 0.126, 0.068	±50	[22]
		Female	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.019, 0.078, 0.130, 0.234, 0.275, 0.372		
	≥1+	Male	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.000, 0.000, 0.018, 0.033, 0.112, 0.077		
		Female	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.003, 0.010, 0.048, 0.079, 0.211, 0.224		
From (3) heart attack to (5) death by sex and age	1st year	Male	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	2.8, 13.4, 13.0, 19.5, 33.7, 33.3	±50	[22]
		Female	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	33.3, 0.0, 16.9, 25.0, 36.6, 45.8		
	2nd year	Male and female	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	3.8, 3.8, 6.7, 19.5, 41.2, 100.0	±50	[24]
From (3) heart attack/(4) stroke to (2) ESRD				0.202	±50	[27]
From (1) screened and/or examined to (4) stroke with no treatment by initial dipstick test result, sex and age	<1+	Male	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.026, 0.139, 0.264, 0.477, 0.738, 0.769	±50	[22]
		Female	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.050, 0.202, 0.357, 0.655, 1.052, 1.540		
		Male	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.014, 0.083, 0.124, 0.271, 0.508, 0.570		
		Female	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	0.034, 0.133, 0.187, 0.382, 0.699, 0.905		
From (4) stroke to (5) death by sex and age	1st year	Male	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	19.1, 14.3, 9.9, 10.6, 12.7, 18.2	±50	[22]
		Female	40–44, 45–54, 55–64, 65–74, 75–84, ≥85	13.6, 14.0, 13.7, 6.8, 14.8, 18.1		
	2nd year	Male	40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80–84, ≥85	6.8, 8.2, 9.5, 12.6, 16.6, 23.3, 37.6, 61.9, 95.1, 100.0	±50	Calculated from Suzuki et al. [25, 26]
		Female	40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80–84, ≥85	5.4, 6.4, 7.5, 9.0, 12.5, 18.4, 26.4, 40.1, 52.6, 71.7		

Table 1 continued

			Base-case value	Range tested in sensitivity analysis (%)	Source
From (1) screened and/or examined to (5) death by sex and age	Male	40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80–84, 85–89, 90–94, 95–99, 100	0.002, 0.003, 0.004, 0.007, 0.010, 0.015, 0.024, 0.042, 0.070, 0.119, 0.196, 0.284, 0.397	±50	[28]
	Female	40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80–84, 85–89, 90–94, 95–99, 100	0.001, 0.001, 0.002, 0.003, 0.004, 0.006, 0.010, 0.019, 0.036, 0.070, 0.132, 0.213, 0.327		
Effectiveness of treatment (%)					
Reduction of transition probabilities from (1) screened and/or examined to (2) ESRD with treatment of CKD			42.1	±50	[20]
Reduction of transition probabilities from (1) screened and/or examined to (3) heart attack with treatment of CKD			71.0	±50	[23]
Reduction of transition probabilities from (1) screened and/or examined to (4) stroke with treatment of CKD			69.3	±50	[23]
Quality of life adjustment					
Utility weight					
(1) Screened and/or examined	Stage 1, stage 2, stage 3, stage 4, stage 5		0.940, 0.918, 0.883, 0.839, 0.798	±20	[31]
(2) ESRD			0.658	±20	[32]
(3) Heart attack			0.771		
(4) Stroke			0.714		
Costing					
Annual cost per person (¥)					
Screening	Dipstick test only, serum Cr assay only, dipstick test and serum Cr		267, 138, 342	±50	Survey of health checkup service providers
Detailed examination			25,000	±50	Expert opinion
CKD treatment	Stage 1, stage 2, stage 3, stage 4, stage 5		120,000, 147,000, 337,000, 793,000, 988,000	±50	Expert opinion
ESRD treatment			6,000,000	±50	[33]
Heart attack treatment	1st year, 2nd year		2,780,000, 179,000	±50	[34]
Stroke treatment	1st year, 2nd year		1,000,000, 179,000	±50	[34]

Decision tree

Figure 1a shows our decision tree comparing a do-nothing scenario with a screening scenario. After the decision node, participants under the do-nothing scenario follow the Markov model shown in Fig. 1b. For those under the screening scenario, three types of screening test are considered: (a) dipstick test to check proteinuria only, (b) serum Cr assay only and (c) dipstick test and serum Cr assay. Other tests such as microalbuminuria and cystatin C [14] are not considered, because they are not available options in the context of this study.

Screened participants are portioned between CKD patients who undergo treatment and those who are left untreated through three chance nodes. The first chance node divides the participants between those who require further examination and those left untreated. Participants with (a) dipstick test only, ≥1+; with (b) serum Cr assay only, ≥stage 3; and with (c) dipstick test and serum Cr assay, either ≥1+ or ≥stage 3, are screened as requiring further examination. Those screened as requiring no further examination follow the Markov model. These are implemented by initial renal function stratum.

The second chance node divides participants screened as requiring further examination into those who seek detailed examination at health care providers and those who avoid any further examination. Its probability is assumed at 40.0% based on the literature [15, 16] and of the opinion of an expert committee set up for the purpose of this study, whose members are acknowledged in the "Acknowledgements" section. Those who avoid further examination follow the Markov model.

The third chance node divides participants who underwent further examination into those who undergo treatment of CKD and those left untreated. We derived these probabilities by initial renal function stratum with a Delphi survey of the expert committee. Regarding the strata of stage 3 CKD, a cut-off value of eGFR (50 ml/min/1.73 m^2) and comorbidity such as hypertension, diabetes and/or hyperlipidaemia are considered in order to depict the difference in clinical practice when recommending start of treatment [17]. We label early stage 3 CKD and advanced stage 3 CKD according to this criterion. Among stage 3 CKD patients, the probability of falling into advanced stage 3 CKD by either eGFR <50 ml/min/1.73 m^2 or having comorbidity is 83.5%, calculated from the Japan Tokutei-Kenshin CKD Cohort 2008. Each value is shown in Table 1. All participants follow the Markov model after their completion of detailed examination.

Markov model

The Markov model consists of five health states: (1) screened and/or examined, (2) ESRD, (3) heart attack, (4) stroke and (5) death. Transitions between these states are indicated by arrows. Although individuals follow various courses other than these five health states and indicated transitions, we model in this way based on available data and literature.

We set the span of staying in each state of the Markov model at 1 year. Annual transition probabilities from (1) screened and/or examined to (2) ESRD with no treatment by the initial renal function stratum are calculated from our database of screened cohort in Okinawa Prefecture [18] for this study, since there is no operational predictive model for progression of CKD to ESRD such as Tangri et al. [19] in Japan. Each value is shown in Table 1. Reductions of these transition probabilities brought about by treatment of CKD

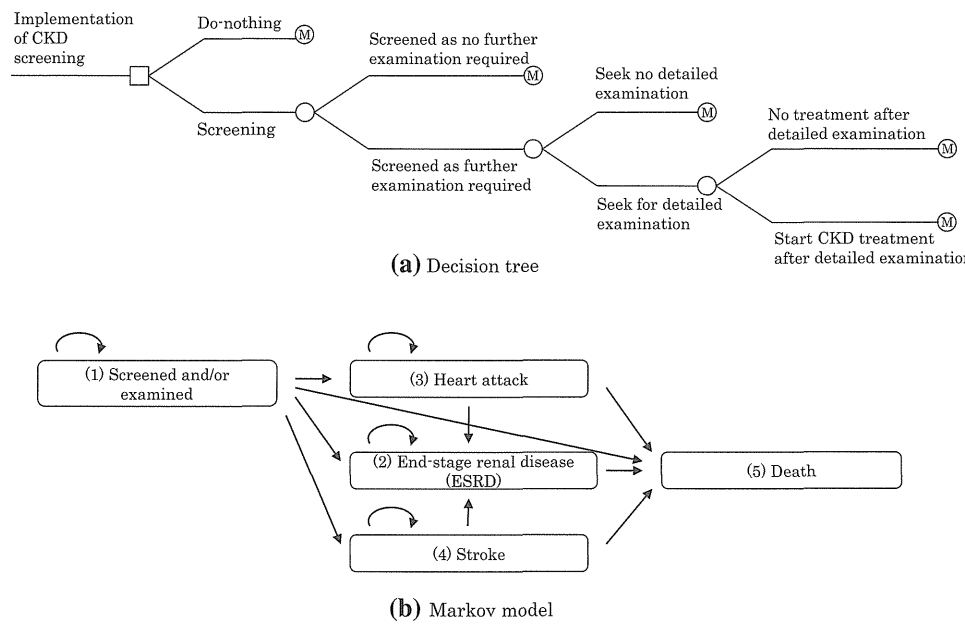

Fig. 1 Economic model. Ⓜ: Markov model