BMJ Open Diabetes Research & Care

Serum levels of mac-2 binding protein are associated with diabetic microangiopathy and macroangiopathy in people with type 2 diabetes

Yoshitaka Hashimoto ,[1] Masahide Hamaguchi ,[1] Ayumi Kaji,[1] Ryosuke Sakai,[1] Noriyuki Kitagawa,[1,2] Michiaki Fukui[1]

To cite: Hashimoto Y, Hamaguchi M, Kaji A, *et al*. Serum levels of mac-2 binding protein are associated with diabetic microangiopathy and macroangiopathy in people with type 2 diabetes. *BMJ Open Diab Res Care* 2020;**8**:e001189. doi:10.1136/bmjdrc-2020-001189

Received 10 January 2020
Revised 4 July 2020
Accepted 18 July 2020

[1]Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, Japan
[2]Department of Diabetology, Kameoka Municipal Hospital, Kameoka, Japan

Correspondence to
Dr Yoshitaka Hashimoto; y-hashi@koto.kpu-m.ac.jp

ABSTRACT

Introduction Non-alcoholic fatty liver disease is reportedly associated with type 2 diabetes and progressive liver fibrosis, as evaluated by transient elastography, and has been linked with micro- and macroangiopathy in people with type 2 diabetes. The purpose of this cross-sectional study was to investigate the association between serum mac-2 binding protein glycosylation isomer (M2BPGi) levels and diabetic complications in people with type 2 diabetes.

Research design and methods Serum M2BPGi levels were measured in terms of cut-off index (C.O.I.) units. Urinary albumin excretion (UAE) was calculated and nephropathy was graded as normoalbuminuria, microalbuminuria, or macroalbuminuria. Retinopathy was divided into three groups: no-diabetic retinopathy (NoDR), non-proliferative-diabetic retinopathy (NPDR), or proliferative-diabetic retinopathy (PDR) .

Results The mean age for the 363 studied subjects (212 males) was 66.4±10.6 years, the median serum M2BPGi level was 0.77 (0.57–1.04) C.O.I., and the median UAE was 22 (9–82.1) mg/g creatinine. M2BPGi levels in microalbuminuria (0.83 (0.61 to 1.18) C.O.I.) and macroalbuminuria (0.88 (0.67 to 1.22) C.O.I.) cases were higher than those in normoalbuminuria cases (0.71 (0.54 to 0.92) C.O.I.). M2BPGi levels in NPDR (0.93 (0.68 to 1.28) C.O.I.) and PDR (0.95 (0.71 to 1.31) C.O.I.) cases were higher than in cases with NoDR (0.73 (0.56 to 0.99) C.O.I.). Furthermore, M2BPGi levels in subjects with a history of cardiovascular diseases were higher than in those with no such history (0.82 (0.65 to 1.22) vs 0.76 (0.55 to 1.03) C.O.I., p=0.019). The logarithm of (M2BPGi+1) was associated with the logarithm of UAE values after adjusting for covariates (standardized β=0.107, p=0.031).

Conclusions This study reveals a close association between serum M2BPGi levels and diabetic microangiopathy and macroangiopathy in people with type 2 diabetes. The results also show that liver fibrosis, evaluated by M2BPGi, is independently associated with an increased risk of albuminuria.

Significance of this study

What is already known about this subject?

- There is an association between non-alcoholic fatty liver disease and microangiopathy and macroangiopathy in people with diabetes.
- Liver fibrosis, evaluated by transient elastography, is associated with microangiopathy and macroangiopathy in people with type 2 diabetes.
- Mac-2 binding protein glycosylation isomer (M2BPGi) has been reported as a non-invasive new serological glycobiomarker for liver fibrosis.

What are the new findings?

- Serum M2BPGi level increased coordinately with diabetic microangiopathy.
- Serum M2BPGi levels in people with a history of cardiovascular diseases were higher than in people without such a history.
- The logarithm of M2BPGi was independently associated with the logarithm of urinary albumin excretion.

How might these results change the focus of research or clinical practice?

- Serum M2BPGi levels could be used to assess diabetic complications.

INTRODUCTION

The number of people afflicted with type 2 diabetes is on the rise, and diabetic complications, such as microangiopathy and macroangiopathy, are important health problems associated with this disease.[1 2] Type 2 diabetes is reportedly associated with non-alcoholic fatty liver disease (NAFLD),[3–5] which includes a range of liver conditions from simple steatosis to fibrosis and cirrhosis.[6] It has been reported that 56% of people with type 2 diabetes have NAFLD, 37% have liver fibrosis, and 17% have severe liver fibrosis.[7]There is an association between NAFLD and microangiopathy[8 9] and macroangiopathy[10] in patients with diabetes. The gold standard for diagnosing liver fibrosis remains liver biopsy. However, liver biopsy is highly invasive. Recently, mac-2 binding protein glycosylation isomer (M2BPGi) has been reported

as a non-invasive new serological glycobiomarker for liver fibrosis.[11–13]

Chronic inflammation and oxidative stress are common pathways in the development of liver fibrosis and diabetes.[14 15] There is an association between progressive liver fibrosis, evaluated by transient elastography, and microangiopathy and macroangiopathy in patients with type 2 diabetes.[16–18] Thus, it is possible that M2BPGi levels are associated with diabetic complications, particularly microangiopathy and macroangiopathy. However, such an association has not been investigated in previous studies. Therefore, in this cross-sectional study of patients with type 2 diabetes, we investigated these associations.

MATERIALS AND METHODS

Participants and study design

The KAMOGAWA cohort study has been performed by us since 2014 on patients with diabetes.[19] Medical data were collected after obtaining informed consent of individuals whose identity was kept secret and compiled into a database. In this cross-sectional study, we selected data of people with type 2 diabetes who attended the outpatient clinic at the Kameoka Municipal Hospital (Kameoka, Japan) or the Kyoto Prefectural University of Medicine (Kyoto, Japan) from January 2016 to May 2018. Data pertaining to the medical history of the participants and their usage of medications were collected. In this study, we only included those people who had their serum levels of M2BPGi checked and excluded those who did not have their urine albumin excretion (UAE) checked.

Biochemical analyses and definitions

The formula used for calculating the body mass index (BMI) was: BMI=weight (kg)/[height (m)]^2. Diagnosis of type 2 diabetes was performed based on the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.[20] The study cohort was divided into non-smokers and current-smokers, based on their declarations in a self-administered questionnaire. Similarly, data of alcohol consumption per week over the past 1 month were collected through a questionnaire. Then, the mean ethanol intake was estimated. The habit of drinking ethanol was defined as an intake greater than 20 g/day.[21] Participants were categorized into non-exercisers or regular exercisers based on their declaration in the questionnaire. Also, data of the activities undertaken by the participants were collected. Regular exercisers were defined as people who performed any type of sports activity at least once a week.[22]

Venous blood was collected from the participants and high-density lipoprotein (HDL) cholesterol, triglycerides, uric acid, creatinine (Cr), aspartate aminotransferase (AST), alanine transaminase (ALT), and platelets were evaluated. Serum M2BPGi levels were evaluated by the M2BPGi reagent and expressed as cut-off index (C.O.I.) units.[23] High-performance liquid chromatography was used to determine hemoglobin A1c levels. The estimated glomerular filtration rate (eGFR) was calculated by the equation of the Japanese Society of Nephrology: eGFR (mL/min/1.73 m^2)=194 × serum Cr$^{-1.094}$ × age$^{-0.287}$ (if women, ×0.739).[24] An immunoturbidimetric method was used for UAE evaluation: UAE (mg/g Cr)=the urinary albumin concentration (mg/L)/the urinary Cr concentration (g/L). The mean value was calculated using three UAE values. The fibrosis-4 (FIB4) index was calculated by age (years)×AST (IU/L)/(platelets (10^9/L)×√ALT (IU/L)).[25] NAFLD fibrosis score was calculated by −1.675 + (0.037×Age (years)) + (0.094×BMI) + (1.13×IFG/diabetes [yes=1, no=0]) + (0.99×AST/ALT) − (0.013×platelet [10^9/L]) − (0.66×albumin (g/dL)).[26]

In this study, diabetic microangiopathy was defined as the presence of diabetic nephropathy and/or retinopathy. The nephropathy stage was defined as follows: UAE less than 30 mg/g Cr was normoalbuminuria, 30–300 mg/g Cr was microalbuminuria, and more than 300 mg/g Cr was macroalbuminuria.[27] Retinopathy was divided into three groups: no diabetic-retinopathy (NoDR), non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR).[28] We defined diabetic macroangiopathy as a history of cardiovascular disease (CVD), such as coronary heart disease, cerebral hemorrhage, or ischemic stroke, which was gathered from the medical records.[29] Acute myocardial infarction, unstable angina, and silent myocardial infarction, but not stable angina pectoris, were included as coronary heart diseases.[29] Patients with an eGFR <60 mL/min/1.73 m^2 were defined as having chronic kidney disease (CKD). After at least a 5 min rest in a quiet space, blood pressures were evaluated twice using a HEM-906 device (Omron Healthcare, Lake Forest, Illinois, USA). We used an average of two values for this study.

Medication data were also collected. Specifically, medications for diabetes, including sodium-glucose cotransporter two inhibitors, glucagon-like peptide-1 receptor agonists, and insulin; medications for hypertension, including renin-angiotensin-aldosterone system (RAAS) inhibitors; and medications for dyslipidemia, including statins were collected.

Statistical analyses

JMP V.13.2 software (SAS Institute, Cary, North Carolina, USA) was used for statistical analyses and GraphPad Prism V.8.4.2 software (GraphPad Software, La Jolla, California, USA) was used for creation figures. Means, medians, and frequencies of variables were calculated. Continuous variables are shown as means±SD or medians (IQR). Categorized variables are shown as a number. $P<0.05$ was set as statistically significant.

Because UAE, duration of diabetes, triglycerides, and M2BPGi were skewed variables, logarithmic transformation was executed before doing correlation, and multiple or logistic regression analyses. Pearson's correlation was calculated to evaluate relationships between logarithms (M2BPGi+1) and other variables. Differences in serum levels of M2BPGi among groups were evaluated by Kruskal-Wallis and Steel-Dwass tests.

Relationships between the logarithm of UAE and logarithm (M2BPGi+1) were determined by multiple regression analyses with the following elements chosen as

independent factors: sex, age, BMI, duration of diabetes, smoking status, systolic blood pressure, hemoglobin A1c, triglycerides, HDL-cholesterol, uric acid, and Cr, and treatment with insulin, RAAS system inhibitors, statins, and platelets.

Last, receiver operator characteristic (ROC) analyses were performed to calculate area under the ROC curve (AUC) of serum levels of M2BPGi for diabetic nephropathy, defined as the presence of microalbuminuria or macroalbuminuria, retinopathy, defined as the presence of NPDR or PDR, and macroangiopathy.

RESULTS

In this study, 365 people with type 2 diabetes were selected. Among these individuals, two for whom there were no UAE data were excluded. Thus, 363 people were enrolled for this study.

Table 1 shows the clinical characteristics data. The mean age was 66.4 (10.6) years, the median serum M2BPGi level was 0.77 (0.57 to 1.04) C.O.I., and the median UAE was 22 (9 to 82.1) mg/g Cr. There were 207 people with normoalbuminuria, 107 people with microalbuminuria, and 49 with macroalbuminuria. Moreover, there were 285 people with NDR, 44 with SDR, and 34 with PDR. Forty-nine people had a history of CVD.

Table 2 reports simple correlations between serum levels of M2BPGi and other factors. Age, duration of diabetes, BMI, systolic blood pressure, Cr level, log UAE, FIB4 index, and NAFLD fibrosis score were positively associated with logarithms (M2BPGi+1), whereas HDL-cholesterol, eGFR, and platelets were negatively associated with logarithms (M2BPGi+1).

Table 3 reports comparisons of the serum levels of M2BPGi in various groups. Insulin usage, retinopathy stage, nephropathy stage, CKD, and history of CVD were associated with higher M2BPGi levels.

Serum levels of M2BPGi in patients with microalbuminuria and macroalbuminuria were higher than in those with normoalbuminuria (figure 1). In addition, serum levels of M2BPGi in patients with NPDR and PDR were higher than in those with NoDR (figure 1).

The results of multiple linear regression analyses of the log UAE values are reported in table 4. The logarithm values of (M2BPGi+1) (standardized β=0.104, p=0.042), HbA1c (standardized β=0.169, p<0.001), Cr (standardized β=0.312, p<0.001), HDL-cholesterol (standardized β=0.137, p=0.004), systolic blood pressure (standardized β=0.144, p=0.002), RAAS inhibitor usage (standardized β=0.138, p=0.005), and exercise habit (standardized β=−0.112, p=0.017) were associated with log UAE values after adjusting for covariates.

The results of ROC and AUC of M2BPGi for diabetic nephropathy, retinopathy and macroangiopathy are shown in figure 2. The optimal cut-off point of M2BPGi for diabetic nephropathy, retinopathy, and macroangiopathy was 0.95, 0.91, and 0.78, respectively.

Table 1 Clinical characteristics of study participants

N	363
Age (years)	66.4 (10.6)
Sex (male/female)	212/171
Family history of diabetes (no/yes)	198/165
Duration of diabetes (year)	12 (6–19)
Height (cm)	160.9 (9.1)
Body weight (kg)	63.1 (12.4)
Body mass index (kg/m^2)	24.3 (4.1)
Systolic blood pressure (mm Hg)	134.1 (18.5)
Diastolic blood pressure (mm Hg)	79.2 (10.9)
Hemoglobin A1c (%)	7.4 (1.3)
Hemoglobin A1c (mmol/mol)	56.9 (14.0)
Triglycerides (mmol/L)	1.2 (0.9–1.9)
HDL-cholesterol (mmol/L)	1.5 (0.4)
Uric acid (μmol/L)	306.3 (74.4)
Creatinine (μmol/L)	72.9 (30.1)
eGFR (mL/min/1.73 m^2)	69.6 (19.1)
Urinary albumin excretion (mg/g creatinine)	22 (9–82.1)
Aspartate aminotransferase (U/L)	21 (17–27)
Alanine transaminase (U/L)	19 (14–28)
Platelets (×10^4/uL)	22.5 (6.2)
FIB4 index	0.68 (0.34–2.52)
NAFLD fibrosis score	2.22 (0.79)
Serum M2BPGi (C.O.I)	0.77 (0.57–1.04)
Nephropathy (normoalbuminuria/ microalbuminuria/macroalbuminuria)	207/107/49
Retinopathy (NoDR/NPDR/PDR)	285/54/24
Chronic kidney disease (no/yes)	256/107
History of cardiovascular disease	315/49
Smoking (non-smoker/smoker)	309/54
Exercise habit (no/yes)	190/173
Habit of drinking alcohol (no/yes)	326/37
SGLT2 inhibitor usage (no/yes)	302/61
GLP-1 analog usage (no/yes)	301/62
Insulin treatment (no/yes)	279/84
RAAS inhibitor usage (no/yes)	201/162
Statin treatment (no/yes)	226/137

Data are expressed as means (SD), medians (IQR), or absolute numbers.
C.O.I, cut-off index; eGFR, estimated glomerular filtration rate; FIB4, fibrosis 4; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; M2BPGi, Mac-2 binding protein glycosylation isomer; NAFLD, non-alcoholic fatty liver disease; NoDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; RAAS, renin-angiotensin-aldosterone system; SGLT2, sodium-glucose cotransporter 2.

DISCUSSION

This study clarifies the relationship between the serum M2BPGi levels and diabetic microangiopathy and macroangiopathy in patients with type 2 diabetes. This is the first study, to our knowledge, to reveal the association between M2BPGi levels and diabetic

Table 2 Correlations between logarithmic (M2BPGi+1) and other variables

Variables	*r*	P value
Age	0.240	<0.001
Logarithmic (duration of diabetes+1)	0.149	0.005
Body mass index	0.116	0.027
Systolic blood pressure	0.118	0.025
Diastolic blood pressure	0.031	0.559
Hemoglobin A1c	0.056	0.287
Logarithmic triglycerides	0.009	0.865
HDL-cholesterol	−0.160	0.002
Uric acid	0.102	0.054
Creatinine	0.129	0.014
eGFR	−0.218	<0.001
Logarithmic UAE	0.216	<0.001
Alanine transaminase	0.072	0.173
Platelets	−0.239	<0.001
FIB4 index	0.122	0.021
NAFLD fibrosis score	0.341	<0.001

Correlations between M2BPGi and other variables were evaluated by Pearson correlations.
eGFR, estimated glomerular filtration rate; FIB4, fibrosis 4; HDL, high-density lipoprotein; M2BPGi, Mac-2 binding protein glycosylation isomer; NAFLD, non-alcoholic fatty liver disease; UAE, urinary albumin excretion.

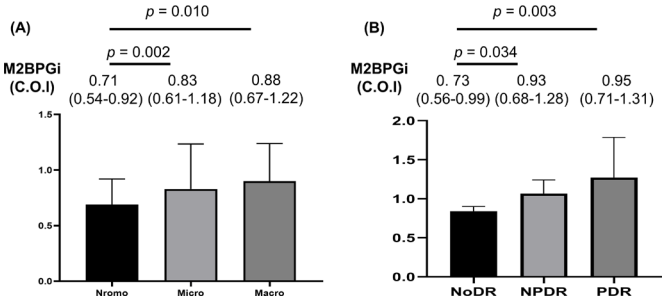

Figure 1 Comparisons of M2BPGi among nephropathy and retinopathy stages. (A) Comparisons of M2BPGi among nephropathy stage. (B) Comparisons of M2BPGi among retinopathy stage. Differences among nephropathy and retinopathy stages were evaluated by a one-way analysis of variance and Tukey honestly significant difference test. C.O.I, cut-off index; M2BPGi, mac-2 binding protein glycosylation isomer; NoDR, no diabetic-retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

microangiopathy and macroangiopathy in people with type 2 diabetes.

Possible interpretations for the connection between M2BPGi levels and diabetic microangiopathy and macroangiopathy are as follows. M2BPGi is a marker of liver fibrosis, although the mechanism of the association between M2BPGi and liver fibrosis is not fully understood.[11] Cross-sectional studies and retrospective cohort study reported that there was an association between progressive liver fibrosis, evaluated by transient elastography, and microangiopathy and macroangiopathy in people with type 2 diabetes.[16–18] In fact, liver fibrosis is a mortality risk from CVD in people with NAFLD.[30]

Both liver fibrosis and diabetic microangiopathy or macroangiopathy are associated with chronic inflammation, including tumor necrosis factor-α (TNF-α), the RAAS, and intercellular adhesion molecule-1. In patients with liver fibrosis, the expression of TNF-α is increased.[31] TNF-α induces renal damage through several mechanisms.[32 33] The cytotoxicity induced by TNF-α stimulates apoptosis of glomerular cells and, consequently, results in the progression of albuminuria[34] and CVD.[32] In addition, TNF-α is associated with diabetic retinopathy through

Table 3 Comparisons of M2BPGi in various groups

	M2BPGi	P value
Sex (male/female)	0.74 (0.56–0.99)/0.83 (0.59–1.12)	0.049
Smoking (non-/current-smoker)	0.78 (0.58–1.08)/0.69 (0.55–0.94)	0.078
Exercise habit (no/yes)	0.82 (0.57–1.07)/0.74 (0.56–1.00)	0.157
Habit of drinking alcohol (no/yes)	0.78 (0.57–1.06)/0.68 (0.52–0.96)	0.076
RAAS inhibitor usage (no/yes)	0.75 (0.55–1.07)/0.78 (0.62–1.01)	0.601
Statins (no/yes)	0.77 (0.57–1.07)/0.78 (0.57–0.99)	0.712
Insulin treatment (no/yes)	0.75 (0.56–1.00)/0.89 (0.62–1.23)	0.015
Nephropathy (normo/micro/macroalbuminuria)	0.71 (0.54–0.92)/0.83 (0.61–1.18)*/0.88 (0.67–1.22)*	<0.001
Retinopathy (NoDR/NPDR/PDR)	0.73 (0.56–0.99)/0.93 (0.68–1.28)†/0.95 (0.71–1.31)†	<0.001
Chronic kidney disease (no/yes)	0.73 (0.54–0.99)/0.88 (0.64–1.23)	<0.001
History of cardiovascular disease (no/yes)	0.76 (0.55–1.03)/0.82 (0.65–1.22)	0.019

Differences among the groups were evaluated by Kruskal-Wallis test.
*p <0.05 vs. normoalbuminuria by the Steel Dwass test.
†p < 0.05 vs. NDR by the Steel Dwass test.
M2BPGi, Mac-2 binding protein glycosylation isomer; NoDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; RAAS, renin-angiotensin-aldosterone system.

Table 4 Multiple linear regression analyses of UAE logarithms

Variables	β	SE	95% CI Lower	95% CI Upper	Standardized β	P value
(Constant)	−4.921	1.250	−7.380	−2.462	–	<0.001
Age	0.017	0.009	0.0002	0.034	0.111	0.048
Female	0.162	0.176	−0.184	0.509	0.066	0.358
Body mass index	0.026	0.022	−0.016	0.069	0.066	0.223
Logarithms (duration of diabetes +1)	0.071	0.115	−0.155	0.297	0.033	0.538
HbA1c	0.215	0.062	0.093	0.336	0.169	<0.001
Creatinine	0.017	0.003	0.011	0.023	0.312	<0.001
Uric acid	0.002	0.001	−0.0007	0.004	0.077	0.155
Logarithms triglycerides	0.329	0.167	−0.004	0.812	0.107	0.052
HDL-cholesterol	0.421	0.206	0.016	0.827	0.137	0.004
Systolic blood pressure	0.012	0.004	0.004	0.021	0.144	0.002
RAAS inhibitor usage	0.460	0.162	0.142	0.778	0.138	0.005
Statins usage	0.024	0.165	−0.301	0.349	0.007	0.885
Insulin treatment	0.224	0.189	−0.148	0.596	0.057	0.238
Exercise habit	−0.371	0.156	−0.676	−0.066	−0.112	0.017
Smoker	0.352	0.218	−0.076	0.780	0.076	0.107
Platelets	−0.004	0.013	−0.031	0.022	−0.016	0.742
Logarithms (M2BPGi+1)	0.708	0.347	0.024	1.391	0.104	0.042

R^2=0.31.
HDL, high-density lipoprotein; M2BPGi, Mac-2 binding protein glycosylation isomer; RAAS, renin-angiotensin-aldosterone system; UAE, urinary albumin excretion.

reduced adherence of leukocytes to retinal blood vessels, blood-retinal barrier breakdown, and apoptosis of retinal cells.[35] Moreover, activation of the RAAS occurs in liver fibrosis,[36] which promotes inflammation through enhanced production of reactive oxygen species leading to hypertrophy and renal fibrosis.[36 37] In addition, RAAS inhibitors are protective effectors for diabetic retinopathy.[38] Furthermore, intercellular adhesion molecule-1 is associated with liver fibrosis,[39] and diabetic microangiopathy[40] and macroangiopathy.[41] Thus, M2BPGi, which is a surrogate marker of liver fibrosis, has a close association with diabetic microangiopathy and macroangiopathy.

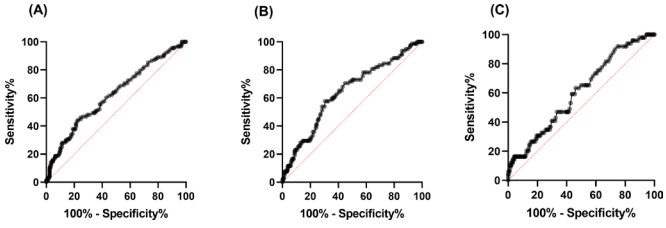

Figure 2 ROC curve and AUC. (A) ROC curve and AUC of M2BPGi for diabetic nephropathy. The optimal cut-off point of the M2BPGi for diabetic nephropathy, defined as the presence of microalbuminuria or macroalbuminuria, was 0.95 (AUC 0.62 (95% CI 0.56 to 0.68), sensitivity=0.44, specificity=0.77, p<0.001). (B) ROC curve and AUC of M2BPGi for diabetic retinopathy. The optimal cut-off point of the M2BPGi for diabetic retinopathy, defined as the presence of non-proliferative diabetic retinopathy or proliferative diabetic retinopathy, was 0.91 (AUC 0.64 (95% CI 0.57 to 0.71), sensitivity=0.58, specificity=0.59, p<0.001). (C) ROC curve and AUC of M2BPGi for diabetic macroangiopathy. The optimal cut-off point of the M2BPGi for diabetic macroangiopathy was 0.78 (AUC 0.60 (95% CI 0.52 to 0.68), sensitivity=0.63, specificity=0.54, p<0.001). AUC, area under the ROC curve; M2BPGi, mac-2 binding protein glycosylation isomer; ROC, receiver operating characteristic.

Previous studies revealed an association between progressive liver fibrosis and diabetic microangiopathy or macroangiopathy in patients with type 2 diabetes.[16–18] To detect liver fibrosis, a liver biopsy is the gold standard; however, this is a highly invasive procedure. M2BPGi is a non-invasive new serological glycobiomarker for liver fibrosis.[11–13] In this study, a history of CVD, duration of diabetes, severity of microangiopathy, systolic blood pressure, HDL-cholesterol, and BMI were associated with the serum levels of M2BPGi. These results were similar to those of previous studies.[42–44] In addition, a recent study revealed that metabolic parameters are associated with an increase of M2BPGi.[45] Furthermore, serum levels of M2BPGi are higher in patients with chronic heart failure.[46]

In this study, we showed that the cut-off points of diabetic nephropathy, retinopathy, and macroangiopathy were 0.95, 0.91, and 0.78, respectively. These cut-off points were lower than that of chronic hepatitis (M2BPGi≥1.00).[11] M2BPGi, which is easy to measure in clinical practice, could be an indicator to prevent future

diabetic microangiopathy or macroangiopathy, and we should pay attention to people with high M2BPGi and improve their metabolic parameters.

The current study has some limitations. First, the study design was cross-sectional. Thus, the causal relationship between the M2BPGi levels and diabetic microangiopathy and macroangiopathy is unclear. Additional prospective studies are needed to establish the association between M2BPGi and the progression or improvement of diabetic microangiopathy or macroangiopathy. Second, we did not perform liver biopsies; thus, we were unable to compare M2BPGi levels with histology data. Third, we did not have liver histology and fibrosis information, nor data on the presence of a hepatitis virus. Such data are important for assessing the degree of liver steatosis and fibrosis and causes of liver diseases. Last, the study population was comprised of only Japanese people; therefore, it is not clear whether the findings of this study can be applied to other ethnic groups.

Despite these limitations, this study reveals, for the first time, the relationship between M2BPGi levels and diabetic microangiopathy and macroangiopathy in people with type 2 diabetes. This study also shows that M2BPGi is independently associated with an increased risk of albuminuria.

Acknowledgements We would like to thank Editage (www.editage.com) for English language editing.

Contributors YH designed the study, researched, analyzed and interpretation data, and wrote manuscript. MH designed the study, researched the data, and contributed to discussion. AK and RS researched and analyzed data and contributed to discussion. NK researched the data and contributed to discussion. MF designed the study, interpreted the data, and contributed to discussion. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests YH received grant support from Asahi Kasei Pharma and honoraria from Mitsubishi Tanabe Pharma Corp and Novo Nordisk Pharma Ltd. MH received grant support from Asahi Kasei Pharma, payment for development of educational presentations from MSD K.K., Mitsubishi Tanabe Pharma Corp, Kowa Co. Ltd. and Sumitomo Dainippon Pharma Co. Ltd., and royalties from US 10,238,714 B2. MF received research supports and payment for development of educational presentation from AstraZeneca plc., Astellas Pharma Inc., Nippon Boehringer Ingelheim Co. Ltd., Daiichi Sankyo Co. Ltd., Eli Lilly Japan K.K., Kyowa Hakko Kirin Co. Ltd., Kissei Pharmaceutical Co. Ltd., MSD K.K., Sumitomo Dainippon Pharma Co. Ltd., Kowa Co. Ltd., Mitsubishi Tanabe Pharma Corp, Novo Nordisk Pharma Ltd., Sanwa Kagaku Kenkyusho Co. Ltd., Sanofi K.K., Ono Pharma Co. Ltd., Taisho Pharma Co. Ltd., Bayer Yakuhin, Ltd., Mochida Pharma Co. Ltd., Johnson & Johnson k.k. Medical Co., Nippon Chemiphar Co. Ltd., Terumo Corp, Teijin Pharma Ltd., and Takeda Pharma Co. Ltd.

Patient consent for publication Not required.

Ethics approval The Ethics Committee of Kyoto Prefectural University of Medicine permitted this study (No. RBMR-E-466–5).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID iDs
Yoshitaka Hashimoto http://orcid.org/0000-0002-8794-0550
Masahide Hamaguchi http://orcid.org/0000-0002-8651-4445

REFERENCES

1 Haffner SM, Lehto S, Rönnemaa T, *et al*. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998;339:229–34.
2 Miyake H, Kanazawa I, Sugimoto T. Albuminuria increases all-cause mortality in Japanese patients with type 2 diabetes mellitus. *J Clin Med* 2018;7:E234.
3 Mantovani A, Byrne CD, Bonora E, *et al*. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. *Diabetes Care* 2018;41:372–82.
4 Okamura T, Hashimoto Y, Hamaguchi M, *et al*. Ectopic fat obesity presents the greatest risk for incident type 2 diabetes: a population-based longitudinal study. *Int J Obes* 2019;43:139–48.
5 Fukuda T, Hamaguchi M, Kojima T, *et al*. Transient remission of nonalcoholic fatty liver disease decreases the risk of incident type 2 diabetes mellitus in Japanese men. *Eur J Gastroenterol Hepatol* 2016;28:1443–9.
6 Matteoni CA, Younossi ZM, Gramlich T, *et al*. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999;116:1413–9.
7 Younossi ZM, Golabi P, de Avila L, *et al*. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. *J Hepatol* 2019;71:793–801.
8 Tripolino C, Irace C, Cutruzzolà A, *et al*. Hepatic steatosis index is associated with type 1 diabetes complications. *Diabetes Metab Syndr Obes* 2019;12:2405–10.
9 Targher G, Bertolini L, Rodella S, *et al*. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. *Diabetologia* 2008;51:444–50.
10 Yan L-H, Mu B, Guan Y, *et al*. Assessment of the relationship between non-alcoholic fatty liver disease and diabetic complications. *J Diabetes Investig* 2016;7:889–94.
11 Kuno A, Ikehara Y, Tanaka Y, *et al*. A serum "sweet-doughnut" protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. *Sci Rep* 2013;3:1065.
12 Shirabe K, Bekki Y, Gantumur D, *et al*. Mac-2 binding protein glycan isomer (M2BPGi) is a new serum biomarker for assessing liver fibrosis: more than a biomarker of liver fibrosis. *J Gastroenterol* 2018;53:819–26.
13 Abe M, Miyake T, Kuno A, *et al*. Association between Wisteria floribunda agglutinin-positive Mac-2 binding protein and the fibrosis stage of non-alcoholic fatty liver disease. *J Gastroenterol* 2015;50:776–84.
14 Diehl AM, Li ZP, Lin HZ, *et al*. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2005;54:303–6.
15 Okuno Y, Fukuhara A, Hashimoto E, *et al*. Oxidative stress inhibits healthy adipose expansion through suppression of SREBF1-Mediated lipogenic pathway. *Diabetes* 2018;67:1113–27.
16 Yeung M-W, Wong GL-H, Choi KC, *et al*. Advanced liver fibrosis but not steatosis is independently associated with albuminuria in Chinese patients with type 2 diabetes. *J Hepatol* 2017. doi:10.1016/j.jhep.2017.09.020. [Epub ahead of print: 06 Oct 2017].
17 Lombardi R, Airaghi L, Targher G, *et al*. Liver fibrosis by FibroScan® independently of established cardiovascular risk parameters associates with macrovascular and microvascular complications in patients with type 2 diabetes. *Liver Int* 2020;40:347–54.
18 Kitagawa N, Hashimoto Y, Hamaguchi M, *et al*. Liver stiffness is associated with progression of albuminuria in adults with type 2 diabetes: nonalcoholic fatty disease cohort study. *Can J Diabetes* 2020;44:428–33.
19 Sakai R, Hashimoto Y, Ushigome E, *et al*. Late-night-dinner is associated with poor glycemic control in people with type 2 diabetes: the KAMOGAWA-DM cohort study. *Endocr J* 2018;65:395–402.
20 American Diabetes Association. 2. classification and diagnosis of diabetes: *Standards of Medical Care in Diabetes-2018*. *Diabetes Care* 2018;41:S13–27.
21 Kaji A, Hashimoto Y, Sakai R, *et al*. Frequent usage of convenience stores is associated with low diet quality. *Nutrients* 2019;11:E1212.

22 Okamura T, Hashimoto Y, Hamaguchi M, *et al*. Short sleep duration is a risk of incident nonalcoholic fatty liver disease: a population-based longitudinal study. *J Gastrointestin Liver Dis* 2019;28:73–81.
23 Yamasaki K, Tateyama M, Abiru S, *et al*. Elevated serum levels of Wisteria floribunda agglutinin-positive human Mac-2 binding protein predict the development of hepatocellular carcinoma in hepatitis C patients. *Hepatology* 2014;60:1563–70.
24 Matsuo S, Imai E, Horio M, *et al*. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53:982–92.
25 Sumida Y, Yoneda M, Hyogo H, *et al*. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012;12:2.
26 Angulo P, Hui JM, Marchesini G, *et al*. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846–54.
27 Hashimoto Y, Tanaka M, Senmaru T, *et al*. Heart rate-corrected QT interval is a novel risk marker for the progression of albuminuria in people with Type 2 diabetes. *Diabet Med* 2015;32:1221–6.
28 Wu H, Hwang D-K, Song X, *et al*. Association between aqueous cytokines and diabetic retinopathy stage. *J Ophthalmol* 2017;2017:9402198.
29 Yoshitaka H, Hamaguchi M, Kojima T, *et al*. Nonoverweight nonalcoholic fatty liver disease and incident cardiovascular disease: a post hoc analysis of a cohort study. *Medicine* 2017;96:e6712.
30 Ekstedt M, Hagström H, Nasr P, *et al*. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015;61:1547–54.
31 Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6:772–83.
32 Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 2006;444:875–80.
33 Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, *et al*. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol* 2011;7:327–40.
34 Navarro JF, Mora C, Gómez M, *et al*. Influence of renal involvement on peripheral blood mononuclear cell expression behaviour of tumour necrosis factor-alpha and interleukin-6 in type 2 diabetic patients. *Nephrol Dial Transplant* 2008;23:919–26.
35 Tang J, Kern TS. Inflammation in diabetic retinopathy. *Prog Retin Eye Res* 2011;30:343–58.
36 Osterreicher CH, Taura K, De Minicis S, *et al*. Angiotensin-converting-enzyme 2 inhibits liver fibrosis in mice. *Hepatology* 2009;50:929–38.
37 Unger T. The role of the renin-angiotensin system in the development of cardiovascular disease. *Am J Cardiol* 2002;89:3–9.
38 Kaštelan S, Tomić M, Gverović Antunica A, *et al*. Inflammation and pharmacological treatment in diabetic retinopathy. *Mediators Inflamm* 2013;2013:213130.
39 Migita K, Horai Y, Kozuru H, *et al*. Serum cytokine profiles and Mac-2 binding protein glycosylation isomer (M2BPGi) level in patients with autoimmune hepatitis. *Medicine* 2018;97:e13450.
40 Khalfaoui T, Lizard G, Ouertani-Meddeb A. Adhesion molecules (ICAM-1 and VCAM-1) and diabetic retinopathy in type 2 diabetes. *J Mol Histol* 2008;39:243–9.
41 Gross MD, Bielinski SJ, Suarez-Lopez JR, *et al*. Circulating soluble intercellular adhesion molecule 1 and subclinical atherosclerosis: the coronary artery risk development in young adults study. *Clin Chem* 2012;58:411–20.
42 Sugiura T, Dohi Y, Takase H, *et al*. Serum levels of Mac-2 binding protein increase with cardiovascular risk and reflect silent atherosclerosis. *Atherosclerosis* 2016;251:192–6.
43 Niinaga R, Yamamoto H, Yoshii M, *et al*. Marked elevation of serum M2BP-adiponectin complex in men with coronary artery disease. *Atherosclerosis* 2016;253:70–4.
44 Arai T, Atsukawa M, Tsubota A, *et al*. Factors influencing subclinical atherosclerosis in patients with biopsy-proven nonalcoholic fatty liver disease. *PLoS One* 2019;14:e0224184.
45 Sugiura T, Dohi Y, Takase H, *et al*. Factors associated with longitudinal changes in serum concentrations of Mac-2 binding protein: a prospective 3-year observational study. *Nutr Metab Cardiovasc Dis* 2019;29:1337–44.
46 Okada A, Kanzaki H, Hamatani Y, *et al*. Increased serum Wisteria floribunda agglutinin positive Mac-2 binding protein (Mac-2 binding protein glycosylation isomer) in chronic heart failure: a pilot study. *Heart Vessels* 2018;33:385–92.