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Urinary Fetuin-A Fragments Predict Progressive Estimated Glomerular Filtration Rate Decline in Two Independent Type 2 Diabetes Cohorts of Different Ethnicities

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Keywords
Diabetic kidney disease · Type 2 diabetes · Biomarker · Estimated glomerular filtration rate decline

Abstract

Introduction: There is a great clinical need for novel markers to predict kidney function decline in patients with type 2 diabetes. We explored the potential of posttranslationally modified fetuin-A fragments in urine (uPTM-FetA) as such a marker. ***Methods:*** We included patients with type 2 diabetes from two independent, nonoverlapping prospective cohort studies. A cut-off for uPTM-FetA, measured via ELISA method, was determined using the Youden index in the primary cohort of patients with type 2 diabetes from Taiwan. Kidney endpoint was defined as an estimated glomerular filtration rate (eGFR) decline ≥30% from baseline, reaching of an eGFR <15 mL/min/1.73 m^2, or a need of renal replacement therapy. Prospective associations were assessed in Cox regression models. All analyses were replicated in a cohort of patients with type 2 diabetes from the Netherlands. ***Results:*** In total, 294 patients with type 2 diabetes (age 61 ± 10 years, 55% male, eGFR 88 ± 16 mL/min/1.73 m^2) were included in the primary cohort. During a follow-up of median 4.6 years, 42 participants (14%) experienced the kidney endpoint. Using the defined cut-off, a high uPTM-FetA was associated with a higher risk of renal function decline ($P_{log\text{-}rank}$ < 0.0001). This association was similar in subgroups depending on albuminuria. This association remained, independent of age, sex, baseline eGFR, albuminuria, HbA1c, and other potential confounders (HR: 9.94; 95% CI: 2.96–33.40;

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$p < 0.001$ in the final model). Analyses in the validation cohort (376 patients with type 2 diabetes, age 64 ± 11 years, 66% male, eGFR 76 ± 24 mL/min/1.73 m^2) using the same cut-off yielded similar results. ***Conclusion:*** uPTM-FetA was independently associated with kidney function decline in patients with type 2 diabetes validated in a 2-cohort study. The significant additive predictive power of this biomarker from conventional risk factors suggests its clinical use for renal function progression in patients with type 2 diabetes.

Introduction

Due to the increasing incidence and prevalence of type 2 diabetes worldwide, diabetic kidney disease (DKD) has become the most common cause of chronic kidney disease and end-stage kidney disease [1]. DKD develops in up to 40% of patients with type 2 diabetes, and its progression may be slowed or prevented through intervention [2]. Therefore, timely identification of patients at high risk of kidney function decline is of utmost importance.

The marker that is generally used in current clinical practice to assess risk of progressing to chronic kidney disease, is the creatinine-based estimated glomerular filtration rate (eGFR) [2]. Unfortunately, eGFR is burdened by confounding factors such as muscle mass, highly variable intra-individual trajectories, and unreliable prediction of progression of kidney disease, particularly in the early stages of DKD [3, 4]. Albuminuria is another commonly used biomarker to assess risk of DKD [2, 5]. However, up to 40% of patients with type 2 diabetes who suffer from renal function worsening remain normoalbuminuric [6, 7]. Thus, due to the low sensitivity of albuminuria, its use in predicting DKD progression is limited.

Consequently, both eGFR and albuminuria are considered insufficient to predict an individual's kidney function decline [3, 4, 6]. Moreover, these measures may not be adequately used to assess response to renoprotective treatments in DKD. A potential explanation for the limited ability of these and other existing markers of kidney function decline, is that they are "late" markers that show kidney injury/dysfunction when the damage has already occurred. Therefore, there is a need for biomarkers closely representing underlying molecular mechanisms involved in disease progression, that can ideally be targeted by therapies. However, at this point, there is a lack of such biomarkers with proven additional predictive value of DKD [8].

Fetuin-A, encoded by *AHSG* gene, is a hepatokine associated with insulin resistance and is an independent risk factor of type 2 diabetes [9, 10]. In an insulin-resistant state, there is a shift of insulin signaling from the PI3K axis to the MAPK axis in endothelial cells of renal arterioles, causing renal vasoconstriction [11]. Fetuin-A has also been reported to stimulate proinflammatory cytokine secretion by perivascular fat tissue, and we can speculate that it causes impairment of renal function by affecting renal sinus fat which is located around renal arteries [12]. Besides the renal vasculature, insulin resistance has a direct impact on podocyte viability and tubular function [13].

As such, we hypothesize that fetuin-A may be a marker that is causally involved and may have added predictive value in the development and progression of DKD. This hypothesis is corroborated by the results of large-scale urine proteomic profiling of among participants from the Taiwan Renal Biomarker Study, in which urinary concentrations of a posttranslationally modified connecting peptide-containing fetuin-A fragments (uPTM-FetA) were identified as a candidate marker of DKD in patients with type 2 diabetes [14, 15]. In the current study, we aimed to evaluate uPTM-FetA as an early marker of kidney function deterioration in two large, independent, prospective cohort studies in patients with type 2 diabetes of two different ethnicities.

Materials and Methods

Study Design

For the current study, we included patients from two independent, nonoverlapping prospective cohort studies including patients with type 2 diabetes from different geographical areas. All analyses were first performed in the primary cohort initiated at the National Taiwan University Hospital (NTUH) in Taiwan. These findings were then independently replicated in a cohort from the DIAbetes and LifEstyle Cohort Twente (DIALECT) in the Netherlands.

Primary Cohort (NTUH)

This prospective cohort study (project title: Validation of Early Diagnostics Biomarkers for Diabetic Nephropathy and its extension; registered project with Institutional Review Board approval No.: 201107004RC and 201705062RIPD) was conducted in patients with type 1 and type 2 diabetes at the NTUH in Taiwan between November 2011 and February 2018. A detailed overview of inclusion and exclusion criteria is provided in online supplementary Table S1 (for all online suppl. material, see https://doi.org/10.1159/000534514). The study was carried out in accordance with the guidelines of good clinical practice and the WMA Declaration of Helsinki. All subjects provided written informed consent prior to study participation.

Validation Cohort (DIALECT)

To validate the optimal cut-off in assessing risk of renal function loss, data from DIALECT cohort study were used, which has been described in detail elsewhere [16]. In brief, DIALECT is a prospective

cohort study in patients with type 2 diabetes, performed in the Ziekenhuisgroep Twente Hospital, which is located in Almelo and Hengelo, the Netherlands. Adult patients with type 2 diabetes treated in the outpatient clinic of this secondary care hospital were eligible and recruited between March 2009 and May 2019 (online suppl. Table S1). The study was performed according to the guidelines of good clinical practice and the WMA Declaration of Helsinki, and all subjects provided written informed consent before study participation. The study protocol was reviewed and approved by the Medical Ethical Committees (METc), METc-Twente (Enschede, the Netherlands) and METc-Groningen (Groningen, the Netherlands), approval numbers NL57219.044.16 and 1009.68020, respectively.

uPTM-FetA Measurements

For both cohorts, spot urine samples were collected at baseline. These samples were stored at −80°C until measurement. uPTM-FetA was measured using the Human uPTM3-DKD ELISA kit (CE-IVD, manufacturer: Bio Preventive Medicine Corp., trade name: DNlite-IVD103). Samples with uPTM-FetA concentrations between assay limit of quantitation and limit of detection (LoD) were set to the LoD, and those below LoD were set to "non-detectable." uPTM-FetA concentrations were divided by urinary creatinine (Ucr) into uPTM-FetA/Ucr for normalization.

Definition of the Kidney Endpoint

Kidney function was assessed using the eGFR calculated with the 2009 creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation for both cohorts. The primary outcome of kidney function deterioration was defined as an eGFR decline ≥30% from baseline [17, 18], reaching eGFR <15 mL/min/1.73 m^2, or need of renal replacement therapy (i.e., kidney transplantation or initiation of dialysis) [17]. Follow-up was censored at the end of follow-up, or after the date that the kidney endpoint was met.

Covariables

At baseline, clinical, anthropometric, and demographic data were retrieved through electronic patient records and/or questionnaires. The severity of albuminuria was determined on the basis of the urinary albumin-to-creatinine ratio (UACR). All other laboratory measurements were performed using routine laboratory methods.

Statistical Analyses

Baseline characteristics were presented as mean ± standard deviation, median [interquartile range], or frequency (percentage), depending on data distribution. We performed receiver operating characteristic analysis and determined a cut-off value for uPTM-FetA/Ucr with optimal sensitivity and specificity using the Youden index by using the data from the primary (NTUH) cohort [19]. According to the established cut-off, patients were categorized into low-risk (uPTM-FetA/Ucr < optimal cut-off) or high-risk (uPTM-FetA/Ucr ≥ optimal cut-off) groups. Statistical significance of differences between the groups were assessed using Student's *t* tests, Mann-Whitney U tests, or Fisher's exact test, depending on data distribution.

Kaplan-Meier curves are presented to visualize the occurrence of the kidney endpoint during follow-up between groups, where log-rank tests were used to assess statistical significance of differences between the groups. The associations of uPTM-FetA/Ucr with risk of eGFR decline were analyzed using Cox proportional-hazards regression analyses. Non-normally distributed covariables were naturally log-transformed prior to analysis. Hazard ratios and 95% confidence intervals were presented per one standard deviation relative increment (risk factors of continuous nature) or per change compared with a reference group (risk factors of categorical nature). In model 1, we performed multivariable-adjusted analyses with traditional risk factors (age, sex, eGFR, UACR, HbA1c). Thereafter, we computed further models with additive adjustments to model 1, including lipid-related parameters (i.e., total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and use of lipid-lowering drugs; model 2); blood pressure-related parameters (systolic blood pressure, diastolic blood pressure, and use of antihypertensive drugs; model 3); other clinical parameters (body mass index, smoking status; model 4); liver function abnormality (model 5); and a combination of all factors above (model 6).

To evaluate the prognostic performance of our biomarker, uPTM-FetA/Ucr, we incorporated it into the clinical plus biomarker model. Various measures were utilized to assess the added value of the biomarker in predicting the risk of kidney function deterioration. Model fit was assessed using the Akaike information criterion (AIC) and the likelihood ratio test (LRT), where lower AIC values or higher χ^2 values indicated superior global fit. Model calibration was evaluated using the Hosmer-Lemeshow goodness-of-fit test, with p values >0.05 indicating good agreement between observed and predicted outcomes. Model discrimination was determined by calculating the area under the curve (AUC) of the receiver operating characteristic. The improvement in AUC after incorporating the biomarker was calculated.

To assess model reclassification, we used continuous/category-free net reclassification improvement (NRI > 0) since there were no established risk cut-offs that justified the use of categorical NRI. The overall NRI measured the proportion of predicted probabilities that shifted upward or downward when the biomarker was added to the clinical model. Additionally, the absolute integrated discrimination improvement (IDI) was employed to quantify the average increase in predicted probabilities for subjects with kidney function deterioration and the reduction for those without, following the addition of the biomarker. All data were analyzed using R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). For all statistical analyses, p value <0.05 was considered significant.

Validation Cohort Analyses

Similar to the primary analyses, patients in the validation cohort (DIALECT) were stratified into high-risk and low-risk groups, based on the uPTM-FetA/Ucr cut-off obtained from NTUH cohort study. Survival analyses including Cox proportional hazard regression models as described for the primary analyses were repeated in this validation cohort, with the same kidney endpoint as outcome variable.

Results

Baseline Characteristics

NTUH Cohort

Among all participants enrolled in the NTUH cohort, 294 were eligible for inclusion in the current study (online suppl. Fig. 1). Mean age was 61 ± 10 years, 163 (55%) were male, eGFR was 88 ± 16 mL/min/1.73 m^2, and median UACR was 9.9 [5.2–25.3] mg/g (Table 1).

Table 1. Baseline characteristics of the NTUH cohort according to uPTM-FetA/Ucr grouping

Variable	Overall (n = 294)	Risk group[1]		p value[2]
		low-risk (n = 112)	high-risk (n = 182)	
Age, years	61.18 (9.86)	59.92 (10.37)	61.96 (9.49)	0.093
Gender (male), n (%)	163 (55)	65 (58)	98 (54)	0.546
Diabetes duration, years	11.00 [7.00, 16.00]	11.00 [7.75, 17.00]	11.00 [7.00, 16.00]	0.879
Baseline eGFR, mL/min/1.73 m^2	88.00 (16.03)	88.97 (16.20)	87.41 (15.93)	0.418
UACR, mg/g	9.90 [5.16, 25.30]	9.02 [4.69, 21.04]	10.95 [5.39, 32.33]	0.105
UACR category, n (%)				0.060
Normal	229 (78)	94 (84)	135 (74)	
Microalbuminuria	65 (22)	18 (16)	47 (26)	
uPTM-FetA/Ucr, ng/mg	9.52 [5.65, 20.34]	4.38 [3.34, 5.95]	16.42 [10.09, 30.19]	<0.001
HbA1c, %	7.32 (1.12)	7.24 (1.08)	7.36 (1.15)	0.388
HDL, mg/dL	46.42 (11.13)	46.49 (11.00)	46.37 (11.24)	0.930
LDL, mg/dL	101.90 (26.77)	107.23 (25.64)	98.62 (26.99)	0.007
SBP, mm Hg	137.51 (18.02)	136.80 (17.59)	137.95 (18.32)	0.594
DBP, mm Hg	81.37 (11.00)	80.91 (10.04)	81.65 (11.57)	0.566
Cholesterol, mg/dL	171.14 (32.94)	176.39 (30.65)	167.91 (33.95)	0.028
Triglyceride, mg/dL	139.99 (93.17)	136.23 (71.37)	142.30 (104.47)	0.555
BMI, kg/m^2	24.79 (3.71)	25.24 (4.14)	24.50 (3.40)	0.114
Glucose AC, mg/dL	141.50 (37.18)	142.77 (39.39)	140.73 (35.85)	0.656
Hypertension medication, n (%)				
Diuretic	15 (5.1)	4 (3.6)	11 (6.0)	0.423
ACEI	5 (1.7)	2 (1.8)	3 (1.6)	>0.999
ARB	142 (48)	50 (45)	92 (51)	0.339
Beta blocker	44 (15)	19 (17)	25 (14)	0.502
CCB	72 (24)	23 (21)	49 (27)	0.264
Lipid medication, n (%)	109 (37)	39 (35)	70 (38)	0.619
Liver function abnormality[3], n (%)	33 (11)	9 (8.0)	24 (13)	0.189

Data are presented as mean ± standard deviation (SD), median [interquartile range (IQR)], or frequency (percentage), depending on data distribution. Non-detectable uPTM-FetAs were set to the lowest value available. eGFR, estimated glomerular filtration rate as calculated using the creatinine-based CKD-EPI formula; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index. [1]uPTM-FetA/Ucr >7.53 ng/mg denotes high-risk group, while uPTM-FetA/Ucr <7.53 ng/mg denotes low-risk group. [2]Welch two-sample t test; Fisher's exact test; Kruskal-Wallis rank sum test. [3]AST/ALT <5 U/L or >40 U/L.

DIALECT Cohort

In total, 376 participants enrolled in the DIALECT cohort were eligible for inclusion in the current study (online suppl. Fig. 1). Mean age was 64 ± 11 years, 249 (66%) were male, eGFR was 76 ± 24 mL/min/1.73 m^2, and median UACR was 9.0 [3.5–47.0] mg/g (Table 2). Baseline eGFR was statistically lower than in NTUH cohort, while UACR was not significantly different (p < 0.001 and p = 0.657, respectively).

Cut-Off Determination and Associations with Kidney Endpoint – NTUH Cohort

During a median follow-up of 4.6 [4.2–4.8] years, 42 participants (14%) from the NTUH cohort reached the kidney endpoint. Based on the Youden index, the optimal uPTM-FetA/Ucr cut-off for kidney endpoint risk stratification was 7.53 ng/mg. Characteristics of the population in strata of uPTM-FetA/Ucr groups are presented in Table 1. In the high-risk uPTM-FetA/Ucr group, 21% experienced the kidney endpoint, which was significantly higher compared to the 3% observed in the low-risk group (p < 0.001; Fig. 1a).

Added Prognostic Value on Top of Current Markers, Including UACR – NTUH Cohort

The associations of the high-risk uPTM-FetA/Ucr group with the kidney endpoint were also observed in univariable Cox regression analyses (HR: 8.94; 95% CI: 2.76–28.90; p < 0.001) and became even more pronounced in the fully adjusted model 6 (HR: 9.94; 95% CI: 2.96–33.40; p < 0.001, online suppl. Table S2). Added value on top of UACR was also illustrated by additional

Table 2. Baseline characteristics of the DIALECT cohort according to uPTM-FetA/Ucr grouping

Variable	Overall[1] (n = 376)	Risk group[1]		p value[2]
		low-risk (n = 223)	high-risk (n = 153)	
Age, years	64.44 (10.75)	62.44 (9.94)	67.36 (11.25)	<0.001
Gender (male), n (%)	249 (66)	131 (59)	118 (77)	<0.001
Diabetes duration, years	13.00 [7.00, 19.00]	13.00 [7.00, 19.00]	13.00 [7.00, 22.00]	0.163
Baseline eGFR, mL/min/1.73 m^2	76.30 (23.98)	81.80 (20.16)	68.28 (26.77)	<0.001
UACR, mg/g	8.99 [3.52, 46.95]	6.80 [3.07, 18.21]	25.31 [5.55, 205.69]	<0.001
uPTM-FetA/Ucr, ng/mg	5.76 [0.76, 17.16]	1.30 [0.06, 3.94]	25.75 [14.24, 48.77]	<0.001
HbA1c, %	7.57 (1.11)	7.57 (1.11)	7.58 (1.11)	0.926
HDL, mg/dL	43.17 (12.33)	44.20 (11.92)	41.67 (12.79)	0.054
LDL, mg/dL	80.96 (32.03)	81.00 (32.81)	80.90 (30.96)	0.976
SBP, mm Hg	131.46 (15.87)	131.45 (14.55)	131.48 (17.66)	0.986
DBP, mm Hg	74.25 (9.82)	74.45 (8.70)	73.95 (11.28)	0.646
Cholesterol, mg/dL	165.35 (39.95)	167.98 (40.12)	161.52 (39.53)	0.123
Triglyceride, mg/dL	218.25 (156.65)	226.76 (173.30)	205.85 (128.14)	0.180
BMI, kg/m^2	32.05 (5.78)	32.21 (5.56)	31.81 (6.08)	0.519
Glucose AC, mg/dL	174.62 (103.55)	171.12 (89.47)	179.71 (121.31)	0.455

Data were presented as mean ± standard deviation (SD), median [interquartile range (IQR)], or frequency (percentage), depending on data distribution. Non-detectable uPTM-FetAs were set to the lowest value available. eGFR, estimated glomerular filtration rate as calculated using the creatinine-based CKD-EPI formula; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index. [1]uPTM-FetA/Ucr >7.53 ng/mg denotes high-risk group, while uPTM-FetA/Ucr <7.53 ng/mg denotes low-risk group. [2]Welch two-sample t test; Fisher's exact test; Kruskal-Wallis rank sum test.

analyses in subgroups depending on uPTM-FetA/Ucr below or above 7.53 ng/mg and UACR below or above 30 mg/g. In the subgroup of patients with UACR <30 mg/g, who would generally be considered at low risk of kidney function deterioration, patients with uPTM-FetA/Ucr >7.53 ng/mg had a higher risk of kidney function deterioration compared to patients with uPTM-FetA/Ucr <7.53 ng/mg (event rates: 17% vs. 3%, respectively; HR: 5.14; 95% CI: 1.54–17.20; p = 0.008). Similarly, in the subgroup of patients with UACR >30 mg/g, who would generally be considered at high risk of kidney function deterioration, patients with uPTM-FetA/Ucr >7.53 ng/mg had a higher risk of kidney function deterioration, whereas there were no events at all among patients with uPTM-FetA/Ucr <7.53 ng/mg (event rates: 35% vs. 0%, respectively; HR: 13.00; 95% CI: 3.76–44.80; p < 0.001; online suppl. Table S3, Fig. S2). In line with these findings, added prognostic value of uPTM-FetA/Ucr on top of a current clinical model including age, sex, eGFR, UACR, and HbA1c was further assessed, as shown in detail in online supplementary Table S4.

The addition of the biomarker to the clinical model improved model fit (ΔLRT χ^2 = 21.85, p < 0.001), calibration (Hosmer-Lemeshow test p = 0.574), discrimination (AUC increase from 0.70 to 0.80, p < 0.001; Fig. 2). The addition of biomarker improves risk classification (continuous NRI 0.72 [95% CI 0.52–0.92]) and also resulting in an overall gain in predictive ability of the model (integrated discrimination improvement 7.8% [5.9–9.8%]).

Results of Validation Study – DIALECT Cohort

In the DIALECT cohort, 125 participants (33%) experienced the kidney endpoint during the median follow-up of 3.7 [2.5–5.3] years. In the high-risk uPTM-FetA/Ucr group, 44% experienced the kidney endpoint, which was higher compared to the 26% observed in the low-risk group (p < 0.001; Fig. 1b).

Discussion

In this study, uPTM-FetA was associated with risk of eGFR decline in patients with type 2 diabetes, and provided significant added prognostic value independent of age, sex, eGFR, UACR, baseline HbA1c, and multiple other potential confounders. These findings were validated in a second, larger cohort of patients with type 2 diabetes, highlighting the potential clinical use of uPTM-FetA as a predictive marker for DKD.

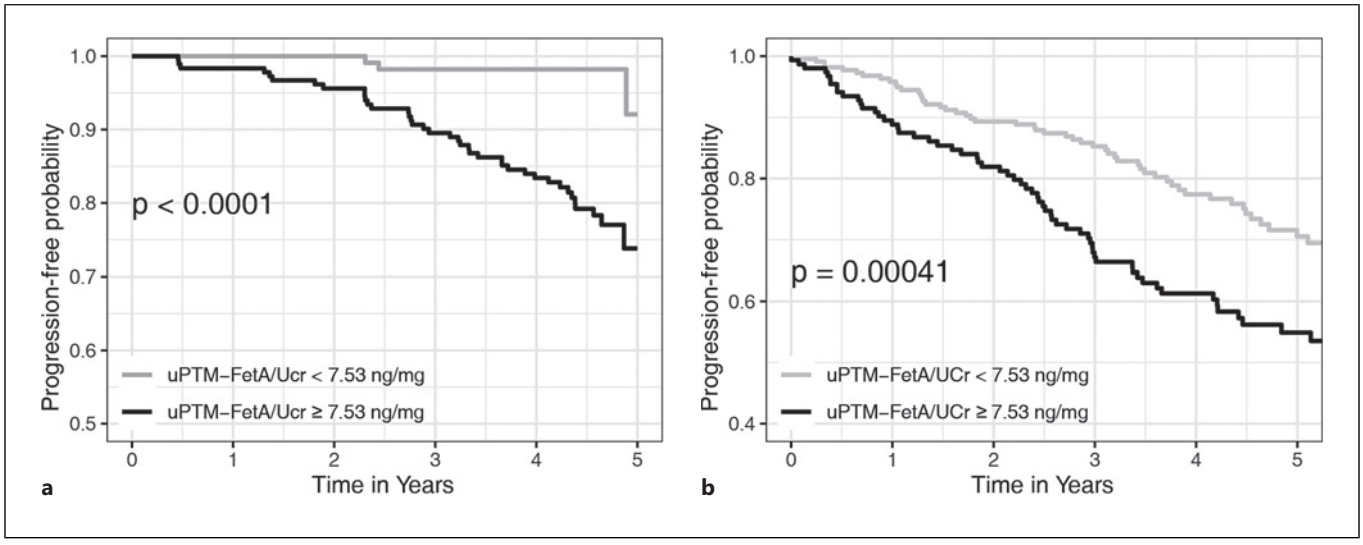

Fig. 1. Kaplan-Meier curves with endpoint of 30% decline or <15 mL/min/1.73 m^2 in eGFR in the high- and low-risk groups stratified by uPTM-FetA/Ucr levels for the NTUH (**a**) and the DIALECT (**b**) cohort.

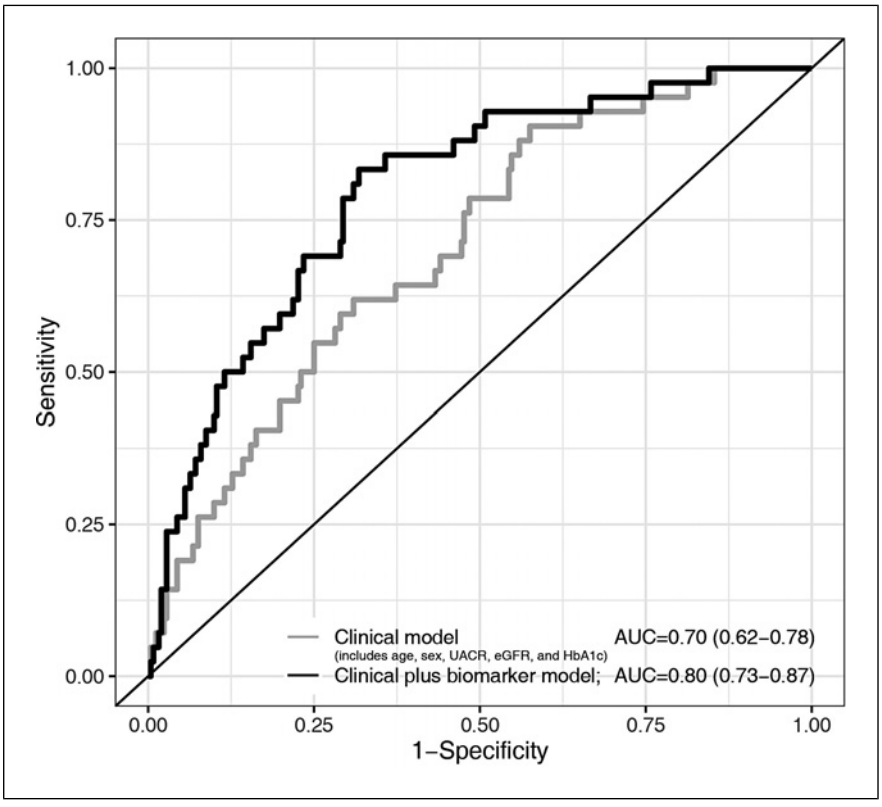

Fig. 2. Increase in performance of predicting kidney function deterioration by adding the biomarker uPTM-FetA/Ucr to the prediction model. The clinical model including age, sex, eGFR, UACR, and HbA1c has an area under the receiver operating characteristic (AUROC) curve of 0.70 (0.62–0.78), while the AUROC curve of the clinical model plus uPTM-FetA/Ucr is 0.80 (0.73–0.87).

The human precursor protein of fetuin-A consists of three parts, namely, the A chain, connecting peptide, and B chain, which are 321, 40, and 27 amino acids in length, respectively [20]. The connecting peptide of the precursor is removed by a posttranslational modification, limited proteolysis, after which only the A and B chain form the active fetuin-A protein [20]. The monoclonal antibody in the ELISA kit that was used in

the current study (Human uPTM3-DKD ELISA kit, BPM Corp.) only detects the connecting peptide-containing fetuin-A.

We found that elevated uPTM-FetA is associated with progression of renal function decline in patients with type 2 diabetes [15]. These findings are generally consistent with previous studies that showed associations of urinary fetuin-A with albuminuria and lower kidney function in patients with type 2 diabetes and DKD [21, 22]. Intriguingly, the concentration of uPTM-FetA is not related to serum PTM-FetA among patients (in-house data from NTUH cohort, data not shown). The incongruence between circulating and urine fetuin-A concentrations was also observed in other studies [23]. This suggests that there may be local production of fetuin-A in the kidney in patients with kidney diseases including DKD.

Fetuin-A is not synthesized by the kidney of human or animal model under healthy conditions [24, 25]. However, a previous study has shown that upon injury, proximal tubule epithelial cells are able to synthesize fetuin-A and release it to the apical side of the tubule [26]. In an animal study using cisplatin-induced and ischemia/reperfusion-induced acute kidney injury rat models, fetuin-A was detected in the urinary exosome fraction instead of in the soluble non-exosomal fraction, indicating that fetuin-A in the urine is produced by the proximal tubule epithelial cells [26]. In addition to that, urinary fetuin-A level increases before serum creatinine surge and also before tubular injury present morphologically [26]. It is hypothesized that local production of fetuin-A by the tubular cells protects the tubules from ongoing inflammation and fibrosis [27]. Whether uPTM-FetA may be merely a predictive marker or also has direct pathogenic effects needs to be further investigated in future preclinical and interventional studies.

Nevertheless, our findings show that in patients with type 2 diabetes, uPTM-FetA was strongly associated with renal function decline, in contrast to UACR in the multivariable-adjusted Cox regression analyses. Moreover, marker has clear added prognostic value on top of a current clinical model including UACR. This suggests that uPTM-FetA may be better suitable for predicting kidney function decline than UACR, or at least provide added clinical value. Moreover, the use of antihypertensives such as angiotensin receptor blockers and angiotensin converting enzyme inhibitors, which are frequently prescribed in patients with type 2 diabetes, reduces albuminuria, thus limiting usefulness of UACR as a predictive marker of DKD. Potentially, uPTM-FetA may not have this disadvantage, although future research is needed to assess the changes in uPTM-FetA after initiation of these antihypertensives.

There are several strengths of this study. The current study included two large, well-characterized cohorts, which allowed for validation and extensive adjustments for potential confounders. Bi-ethnic cohorts including patients from different geographical areas, are reassuring with regard to the external validity of these findings to other type 2 diabetes populations worldwide. In addition, despite the difference between eGFR baseline statuses in these two cohorts, the prognostic ability of uPTM-FetA remained significant. Thus, this biomarker may be potentially useful in patients with type 2 diabetes in various conditions. Our study also has some limitations. The follow-up time in the validation cohort is considerably shorter than that in the primary cohort. Also, due to the observational design of the current study, we are only able to assess the prognostic abilities of uPTM-FetA and cannot confirm any causal effects on kidney disease progression. Moreover, the current study could not assess how other markers such as kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin compare to uPTM-FetA, even though this would provide helpful information on its meaning and interpretation. Finally, none of the patients used SGLT2-inhibitors at baseline, and only a small fraction of the included patients started SGLT2-inhibitor use during the follow-up period. Although the use of these drugs is not expected to change the added value of uPTM-FetA/Ucr in predicting eGFR decline in patients with type 2 diabetes, this may be confirmed in future studies in which larger numbers of patients are using SGLT2-inhibitors.

In conclusion, this study demonstrates that in two large, independent, bi-ethnic prospective cohorts of patients with type 2 diabetes, uPTM-FetA is strongly associated with renal function deterioration, independent of albuminuria, eGFR, age, sex, and multiple other risk factors. These findings were validated in a large, independent, second cohort, which is promising with regard to the potential scientific and clinical use of uPTM-FetA in type 2 diabetes.

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Statement of Ethics

The NTUH study protocol was approved by the Institutional Review Board of the National Taiwan University Hospital Research Ethics Committee (NTUH, Taipei, Taiwan), approval numbers 201107004RC and 201705062RIPD. All subjects provided written informed consent prior to study participation. The DIALECT study protocol was reviewed and approved by the Medical Ethical Committees (METc), METc-Twente (Enschede, the Netherlands) and METc-Groningen (Groningen, the Netherlands), approval numbers NL57219.044.16 and 1009.68020, respectively. All subjects provided written informed consent prior to study participation.

Conflict of Interest Statement

The authors of this manuscript have conflicts of interest to disclose. D.K., S.J.L.B., and L.-M.C. received travel reimbursement from the Bio Preventive Medicine Corp. for attending World Congress of Nephrology 2023. C.-H.H. and T.-L.T. are full-time employees and hold stocks at Bio Preventive Medicine Corp., the company that developed the assay to measure uPTM-FetA in the current study.

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Author Contributions

G.-T.C., D.K., T.-L.T., S.J.L.B., and L.-M.C. contributed to the study design. C.-H.H. and G.-T.C. were responsible for data analysis. G.-T.C., D.K., C.-H.H., and F.F.A. drafted the initial manuscript. T.-L.T., C.-H.L., G.D.L., S.J.L.B., and L.-M.C. provided critical feedback during data analyzing and manuscript drafting. Final manuscript was approved by all authors.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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