Urinary Liver-Type Fatty Acid–Binding Protein and Progression of Diabetic Nephropathy in Type 1 Diabetes

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OBJECTIVE—Diabetic nephropathy (DN) has mainly been considered a glomerular disease, although tubular dysfunction may also play a role. This study assessed the predictive value for progression of a tubular marker, urinary liver-type fatty acid–binding protein (L-FABP), at all stages of DN.

RESEARCH DESIGN AND METHODS—At baseline, 1,549 patients with type 1 diabetes had an albumin excretion rate (AER) within normal reference ranges, 334 had microalbuminuria, and 363 had macroalbuminuria. Patients were monitored for a median of 5.8 years (95% CI 5.7–5.9). In addition, 208 nondiabetic subjects were studied. L-FABP was measured by ELISA and normalized with urinary creatinine. Different Cox proportional hazard models for the progression at every stage of DN were used to evaluate the predictive value of L-FABP. The potential benefit of using L-FABP alone or together with AER was assessed by receiver operating characteristic curve analyses.

RESULTS—L-FABP was an independent predictor of progression at all stages of DN. As would be expected, receiver operating characteristic curves for the prediction of progression were significantly larger for AER than for L-FABP, except for patients with baseline macroalbuminuria, in whom the areas were similar. Adding L-FABP to AER in the models did not significantly improve risk prediction of progression in favor of the combination of L-FABP plus AER compared with AER alone.

CONCLUSIONS—L-FABP is an independent predictor of progression of DN irrespective of disease stage. L-FABP used alone or together with AER may not improve the risk prediction of DN progression in patients with type 1 diabetes, but further studies are needed in this regard.

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Diabetic nephropathy (DN) affects ~30% of all patients with type 1 diabetes. It is also the most severe diabetes complication because it is associated with progression to end-stage renal disease (ESRD) and a high risk of premature death (1,2).

Early screening and detection is essential for the prevention of DN and is currently based on the measurement of the urinary albumin excretion rate (AER) (3). An increased AER is regarded as a marker of glomerular injury, and its early diagnosis makes intervention possible before renal function starts to decline, as reflected by an impaired glomerular filtration rate (GFR). However, AER has some limitations, at both the early and the late stages of disease (4–6).

Although DN has long been considered a glomerular disease, tubulointerstitial injury has also been demonstrated to play a role in the pathogenesis (7). In this context, it is attractive to study molecules that are linked to tubular dysfunction. These molecules may serve as potential new markers for DN and may also provide additional information about clinical course or prognosis that may enable an earlier diagnosis and means to better tailor the treatment.

Urinary liver-type fatty acid–binding protein (L-FABP) is mainly regarded as a urinary tubular biomarker associated with structural and functional kidney damage (8). Urinary levels of L-FABP are not influenced by its serum levels because urinary L-FABP originates mainly from the tubular cells (9). This biomarker is elevated in the early stages of diabetes but is also influenced by lipid-lowering medication and angiotensin II receptor antagonists (10–12). Urinary L-FABP predicts adverse outcomes in acute kidney injury and progression of chronic kidney disease of nondiabetic causes (13–15). It is of note that urinary L-FABP has been linked to DN in patients with type 2 diabetes and has furthermore been suggested to be a predictor of progression to microalbuminuria in patients with type 1 diabetes (16,17). However, whether L-FABP would be a more sensitive marker of DN than AER or whether its predictive role is solely confined to the progression of the disease process is not yet known. Therefore, the aim of the current study is to investigate if baseline levels of L-FABP predict the development of DN and its progression at any stage of the disease and if the use of L-FABP alone or together with AER adds a benefit compared with current standard testing by AER.

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RESEARCH DESIGN AND METHODS

Study sample

This study is part of the ongoing Finnish Diabetic Nephropathy Study (FinnDiane). The study protocol has been described elsewhere and approved by the local ethics committees of all participating centers (18). Written informed consent was obtained from each patient, and the study was performed in accordance with the Declaration of Helsinki.

Blood and urine samples for the current study were collected at baseline for patients who were enrolled between January 1998 and December 2002 and stored at −20°C until 2008. Patients were monitored for a median of 5.8 years (95% CI 5.7–5.9), and clinical outcomes were ascertained. After patients with ESRD were excluded, 1,886 patients remained in the study. The control group comprised nondiabetic subjects without a first- or second-degree relative with kidney disease or diabetes.

Cohort characteristics

Baseline data on medication and diabetes complications were registered with the use of a standardized questionnaire, which was completed by the attending physician using information from the medical files.

Blood pressure, height, weight, and waist-to-hip ratio (WHR) were assessed. Blood was drawn for measurement of HbA_{1c}, lipids, and cystatin C. Assessment of biochemical variables has been described elsewhere (19).

Urinary L-FABP was quantified, in a single 24-h urine collection, using a research L-FABP Elecsys assay on the Cobas Elecsys 411 Immunoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). To determine L-FABP in urine, human urine samples were automatically treated with an alkaline pretreatment that causes the denaturation of proteins in the sample. A biotinylated monoclonal antibody (capture antibody), combined with a ruthenium-labeled monoclonal antibody (detection antibody), reacted with the antigen to form a sandwich complex. After addition of streptavidin-coated beads, this complex became bound to the beads via interaction of biotin and streptavidin.

This mixture was aspirated into the measuring cell, where the beads were magnetically captured onto the surface of the electrode. Emission of photons derived from chemiluminescent reaction was measured by a photomultiplier. The assay demonstrated repeatability below 7% coefficient of variation and a recovery in serial measurements of ~100 ± 10%. The lower detection limit of the assay was determined (<0.1 ng/mL), and no cross-reactivity was observed for the other FABP types. For evaluation, the resulting urinary L-FAB *P* values were normalized with urinary creatinine.

Renal status was defined based on the AER in at least two of three timed urine collections. Patients were divided by AER categorically into those with normal AER (<30 mg/24 h or <20 μg/min), microalbuminuria (30–300 mg/24 h or 20–200 μg/min), and macroalbuminuria (>300 mg/24 h or >200 μg/min). Presence of ESRD was defined according to whether patients were undergoing dialysis or had received a kidney transplant (patients with ESRD were excluded at baseline). The GFR was estimated with a formula based on cystatin C (20).

During follow-up, all patients were managed by their own practitioner and diabetes team, without any attempt to standardize care.

Ascertainment of outcomes

Progression of DN was defined as the passage from one stage to the next based on AER thresholds. ESRD was defined as the requirement of dialysis or kidney transplantation and was identified via a search of the renal registries or center databases and verified from medical files.

Statistical analysis

Normally distributed variables are presented as mean ± SD. Variables nonnormally distributed are presented as median and interquartile range. Comparison between the groups was performed by one-way ANOVA for normally distributed variables and by Mann-Whitney *U* test for nonparametric distributions. Categorical variables were compared between the groups using the χ^2 test.

Cox proportional hazards models were used to analyze the values of L-FABP as an explanatory variable for progression of DN. Separate Cox proportional hazards models were constructed to predict progression at the various stages of DN. The basic models of progression were built by starting with all known risk factors for DN. All of the single covariates were first tested in univariate analysis, and only the significant ones were selected for further analysis. The sets of significant covariates from the univariate analysis were tested in the Cox regression proportional hazards models by using a backward selection algorithm. The variables retained in the models after backward selection constituted the final basic models. Then L-FABP or AER were included in these basic models. Finally, both L-FABP and AER were included in the models. We tested for interaction between variables included in the basic model, but no significant interaction was detected.

The models were also compared using time-dependent receiver operating characteristic (ROC) curve analysis to assess the clinical benefit of using L-FABP, alone or on top of the current clinical standard (AER), as a predictor of DN progression at any stage of the disease.

To see if treatment influenced the results, we performed a supplementary analysis adjusting the models for medications that have been shown to influence urinary L-FABP and AER concentration, including ACE inhibitors, angiotensin II receptor blockers, and any antihypertensive medication, as well as lipid-lowering treatment (21). *P* values < 0.05 were considered statistically significant. The data analysis was performed using MedCalc 12.1.3.0 software (MedCalc Software BVBA, Mariakerke, Belgium) and SPSS 19.0. software (IBM Corporation, Armonk, NY).

RESULTS

Cohort characteristics

Baseline characteristics (Table 1) were used to divide the 2,454 patients with type 1 diabetes into three groups: 1,549 with normal AER, 334 with microalbuminuria, and 363 with macroalbuminuria. In addition, 208 nondiabetic subjects served as the control group. Patients were monitored for 5.8 years (95% CI 5.7–5.9). During the follow-up period, 112 patients with type 1 diabetes progressed from normal AER to microalbuminuria, 46 progressed from microalbuminuria to macroalbuminuria, and 78 progressed from macroalbuminuria to ESRD. The clinical baseline characteristics of progressors and nonprogressors, for all stages of DN, are described in Supplementary Table 1. Progressors from normal AER to microalbuminuria had higher BMI, systolic blood pressure, diastolic blood pressure, HbA_{1c}, total cholesterol, LDL cholesterol, triglycerides, and AER. Patients who progressed from

Table 1—*Clinical baseline data for subjects enrolled in the study*

Variable	Healthy control subjects n = 208	Patient groups: Normoalbuminuric n = 1,549	Microalbuminuric n = 334	Macroalbuminuric n = 363
Sex				
Males	106	732	195	199
Females	102	817	139	164
Age (years)	35.9 ± 11.3	36.2 ± 12.3	38.8 ± 12.7	41.8 ± 10.5
Age of onset (years)	—	17.4 ± 9.3	13.0 ± 9.1	12.5 ± 8.5
Duration (years)	—	18.8 ± 11.7	25.7 ± 11.1	29.3 ± 8.1
BMI (kg/m^2)	24.0 ± 3.0	24.9 ± 3.5	25.6 ± 3.6	26.2 ± 4.1
WHR				
Males	0.92 ± 0.06	0.89 ± 0.07	0.92 ± 0.07	0.94 ± 0.07
Females	0.83 ± 0.05	0.80 ± 0.06	0.83 ± 0.07	0.84 ± 0.07
Smoking history (%)	22.3	41.2	52.4	60.4
Blood pressure (mmHg)				
Systolic	126 ± 15	130 ± 16	136 ± 17	143 ± 20
Diastolic	77 ± 9	78 ± 9	81 ± 10	83 ± 10
HbA_{1c} (%)	5.5 ± 0.4	8.2 ± 1.4	8.8 ± 1.5	9.0 ± 1.6
Cholesterol (mmol/L)				
Total	4.75 ± 0.88	4.80 ± 0.90	4.97 ± 0.88	5.39 ± 1.09
HDL	1.55 ± 0.33	1.35 ± 0.37	1.30 ± 0.39	1.21 ± 0.37
LDL	2.76 ± 0.82	2.95 ± 0.81	3.08 ± 0.80	3.39 ± 0.89
Triglycerides (mmol/L)	0.90 (0.84– 0.97)	0.94 (0.92–0.97)	1.08 (1.02–1.14)	1.36 (1.27–1.46)
AER (mg/24 h)	3 (2–3)	8 (7–8)	50 (43–58)	453 (371–584)
eGFR (mL/min/1.73 m^2)	111 ± 36	101 ± 24	90 ± 24	60 ± 40
L-FABP (μg/μmol)	0.014 (0.008–0.020)	0.039 (0.036–0.044)	0.091 (0.074– 0.107)	0.504 (0.426–0.643)

Categorical data are presented as numbers, and continuous data are presented as mean ± SD, median (interquartile range), or percentage.

microalbuminuria to macroalbuminuria more often had a history of smoking and higher WHR, diastolic blood pressure, HbA_{1c}, total cholesterol, triglycerides, and AER. Patients who progressed from macroalbuminuria to ESRD had higher systolic blood pressure, total cholesterol, triglycerides, and AER and lower estimated GFR (eGFR).

Levels of L-FABP were significantly higher ($P < 0.001$) in patients with type 1 diabetes and normal AER (0.075 μg/μmol) than in nondiabetic subjects (0.014 μg/μmol). Urinary L-FABP levels increased in parallel with worsening stage of DN (Fig. 1*A*). L-FABP was higher in the progressors than in nonprogressors at any stage of DN (Fig. 1*B*).

Progression from normal AER to microalbuminuria

Univariate analysis showed L-FABP predicted the progression from normal AER to microalbuminuria with a hazard ratio (HR) of 4.10 (95% CI 2.31–7.27; $P < 0.001$). To analyze this association in more detail, we used a backward selection procedure to create a Cox regression model out of all of the other potential risk factors as described in RESEARCH DESIGN AND METHODS. The variables that remained in the basic model were: WHR, history of smoking, HbA_{1c}, and total cholesterol. When we included L-FABP in this Cox regression model, L-FABP remained significant (3.22 [1.74–5.95], $P < 0.001$). Finally, when we added AER to the model, L-FABP still remained an independent predictor of progression to microalbuminuria (2.97 [1.49–5.89], $P = 0.002$). AER as a single variable was then added alone to the basic model and together with L-FABP predicted progression to microalbuminuria in all three analyses (Table 2).

When we assessed the potential benefit of using L-FABP instead of AER for the prediction of progression with ROC curve analyses adjusted for the basic model, we found that the area under the curve (AUC [95% CI]) for L-FABP ($AUC_{L\text{-}FABP}$) was smaller than the AUC for AER (AUC_{AER}) at 0.735 (0.711–0.757) vs. 0.778 (0.756–0.799; $P < 0.001$), suggesting that AER performs better. When both urinary biomarkers where included in the model, the AUC of L-FABP plus AER ($AUC_{L\text{-}FABP\&AER}$) was 0.786 (0.765–0.807), which was not significantly larger (Δ_{AUCs} 0.008, $P = 0.09$) then the AUC_{AER} (0.778 [0.756–0.799]) in patients with type 1 diabetes and normal AER (Fig. 2; Supplementary Table 2).

Progression from microalbuminuria to macroalbuminuria

In microalbuminuric patients, univariate analysis (HR [95% CI]) showed that L-FABP is a predictor of progression to macroalbuminuria (1.49 [1.20–1.85], $P < 0.001$). To show that L-FABP is independent from other risk factors, a basic model of progression to macroalbuminuria was built and comprised WHR, HbA_{1c}, and triglycerides. L-FABP remained an independent predictor of progression to macroalbuminuria (1.40 [1.10 – 1.79], $P = 0.006$) when it was added to the basic model. We also wanted to see if L-FABP is independent of AER and added AER to the previous model. Even in this model, L-FABP was an independent predictor of progression to macroalbuminuria (0.673 [0.476–0.954], $P = 0.026$). As expected, AER predicted the progression to macroalbuminuria in all models (Table 2).

We used ROC analysis to assess the potential benefit of using L-FABP instead

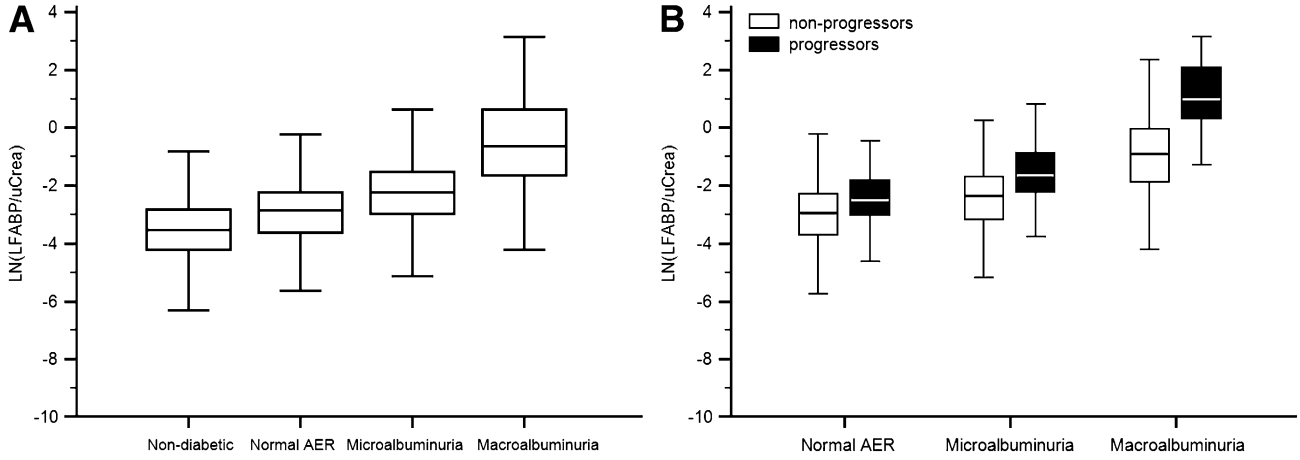

Figure 1—A: *Urinary L-FABP levels across study groups at baseline. The L-FABP levels were significantly different among the study groups. Significant differences* ($P < 0.001$) *in L-FABP levels were observed between the macroalbuminuria group and all other groups. L-FABP levels in the microalbuminuria group were significantly different* ($P < 0.001$) *from healthy patients and those with type 1 diabetes and normal AER. Patients with type 1 diabetes and normal AER had significantly* ($P < 0.001$) *higher L-FABP levels than healthy patients.* B: *Urinary L-FABP levels across study groups at baseline in relation with progression status. L-FABP level is significantly higher* ($P < 0.001$) *for progressors across all groups (normal AER, microalbuminuria, and macroalbuminuria) compared with nonprogressors. The horizontal line in the middle of each box indicates the median; the top and bottom borders of the box mark the 75th and 25th percentiles, respectively, and the whiskers mark the 90th and 10th percentiles.*

of AER. When we compared the AUCs of each marker used on top of the basic progression model, AUC_{AER} was slightly larger than $AUC_{L\text{-}FABP}$ (0.847 [95% CI 0.803–0.898] vs. 0.777 [0.728–0.821], $P = 0.034$), suggesting that AER is a better predictor of progression to macroalbuminuria. When we analyzed whether the concomitant use of both biomarkers added benefit compared with AER alone, we found that there was no difference between $AUC_{AER\&L\text{-}FABP}$ and AUC_{AER} ($P = 0.40$; Fig. 2; Supplementary Table 2).

Progression to ESRD in macroalbuminuric patients

Unadjusted L-FABP predicted the progression to ESRD (HR 1.24 [95% CI 1.19–1.28], $P < 0.001$) in univariate Cox regression analysis. The basic model of progression to ESRD included eGFR and triglycerides. When we added L-FABP to this model, it was independent of the other covariates (1.20 [1.14–1.25], $P < 0.001$). When we further adjusted the model for AER, L-FABP remained an independent predictor of progression to ESRD (1.16 [1.10–1.23], $P = 0.023$; Table 2).

ROC curve analysis revealed that there was no difference between AUC_{AER} and $AUC_{L\text{-}FABP}$ ($\Delta_{AUCs} = 0.011$, $P = 0.280$). Also, when we compared the use of L-FABP together with AER for the prediction of progression to ESRD, the difference between $AUC_{L\text{-}FABP\&AER}$ and AUC_{AER} was nonsignificant ($\Delta_{AUCs} = 0.002$, $P = 0.819$; Fig. 2; Supplementary Table 2).

Effect of treatment on prediction of progression

When we adjusted the L-FABP findings for the use of medication, the results were still significant for all tested medication (data not shown), except for ACE inhibitors (HR 0.773 [95% CI 0.540–1.107], $P = 0.161$) or any antihypertensive medication (0.759 [0.524–1.100], $P = 0.147$), at the stage of microalbuminuria.

CONCLUSIONS—To our knowledge, this is the first study in type 1 diabetes to show that L-FABP is an independent

Table 2—*Prediction of progression using Cox regression analysis with baseline data for L-FABP and AER*

	Unadjusted (univariate)		Adjusted for basic model		Adjusted for basic model and AER	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Normoalbuminuria						
AER (mg/24 h)	1.0159 (1.0131–1.0187)	<0.0001	1.0155 (1.0120–1.0189)	<0.0001	1.0149 (1.0115–1.0184)	<0.0001
L-FABP (μg/μmol)	4.1006 (2.3103–7.2783)	<0.0001	3.2215 (1.7413–5.9597)	0.0002	2.9706 (1.4961–5.8982)	0.0020
Microalbuminuria						
AER (mg/24 h)	1.0061 (1.0048–1.0074)	<0.0001	1.0075 (1.0053–1.0097)	<0.0001	1.0113 (1.0074–1.0152)	<0.0001
L-FABP (μg/μmol)	1.4912 (1.2008–1.8517)	0.0003	1.4061 (1.1029–1.7926)	0.0062	0.6733 (0.4756–0.9533)	0.0265
Macroalbuminuria						
AER (mg/24 h)	1.0005 (1.0004–1.0005)	<0.0001	1.0003 (1.0002–1.0004)	<0.0001	1.0001 (1.0000–1.0003)	0.0225
L-FABP (μg/μmol)	1.2410 (1.1963–1.2874)	<0.0001	1.2001 (1.1442–1.2586)	<0.0001	1.1686 (1.1045–1.2365)	<0.0001

Basic models for progression for every stage are described in RESEARCH DESIGN AND METHODS.

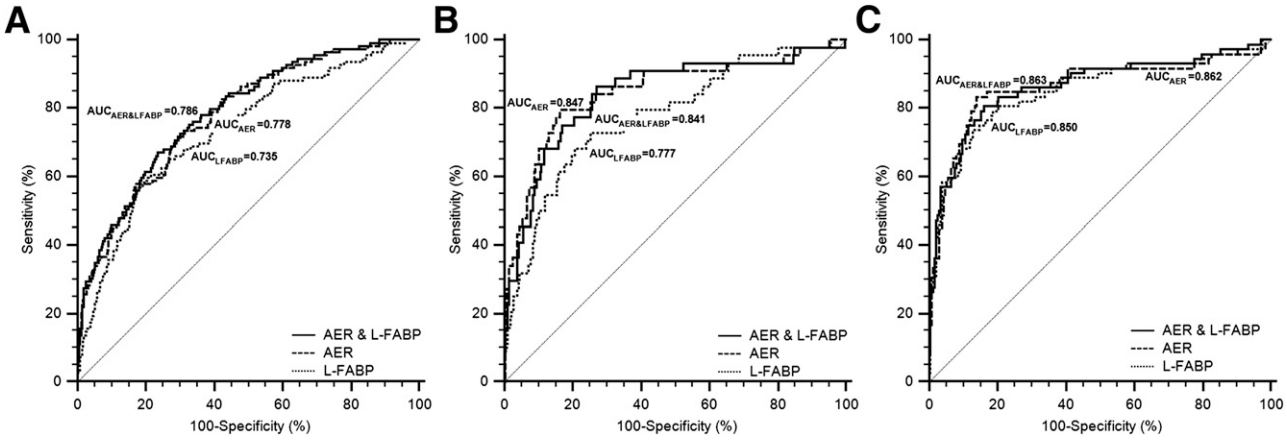

Figure 2—A: *ROC curve analysis for L-FABP and AER in patients with type 1 diabetes and normal AER showed a trend toward an improvement of the risk prediction* (P = *0.09*) *for L-FABP used together with AER* ($AUC_{L\text{-}FABP\&AER}$ = *0.786*) *compared with AER used alone* (AUC_{AER} = *0.778*) *in patients with type 1 diabetes and normal AER.* B: *ROC curve analysis for L-FABP and AER in the microalbuminuria group found no significant difference between* AUC_{AER} (*0.847*) *and* $AUC_{L\text{-}FABP\&AER}$ (*0.841*). $AUC_{L\text{-}FABP}$ (*0.777*) *was significantly smaller than* AUC_{AER} (P = *0.034*). C: *ROC curve analysis for L-FABP and AER in the macroalbuminuria group found no significant difference between* AUC_{AER} (*0.862*) *and* $AUC_{L\text{-}FABP\&AER}$ (*0.863*). $AUC_{AER\&L\text{-}FABP}$ *was significantly larger* (P = *0.012*) *than* $AUC_{L\text{-}FABP}$ (*0.850*).

predictor of progression across all stages of DN. Another interesting finding of this study is that the use of L-FABP together with AER may not improve the risk prediction of DN progression in patients with type 1 diabetes.

The finding that L-FABP is a predictor of progression in patients with type 1 diabetes and normal AER has been suggested earlier, but that study did not have the power to show a predictive value of L-FABP as a continuous variable (17). Our study demonstrates the predictive value of L-FABP not only in patients with type 1 diabetes and normal AER but also across all stages of DN. This may represent an important result, because L-FABP is closely associated with structural and functional tubular kidney damage, and for patients with AER in the "normal" range, we still have no other biomarker or algorithm to identify those at risk for progression to microalbuminuria (10,22).

The ROC curve analysis, however, did not show any benefit of using L-FABP to predict progression to a higher stage, most likely because the progression of DN from microalbuminuria to macroalbuminuria in this study was defined by change in AER. Using an AER definition of progression makes it very difficult for any other variable to outperform the gold standard, the AER. Although recent studies have challenged the classification based on AER, the AER is still useful at the early stages before any decline in GFR occurs and mirrors the progression of more than 70% of patients with DN (6,23). Another option to define progression could be based on change in GFR. This may better reflect the final outcomes compared with AER but might not give enough information at the early stages of DN. This approach was used to define progression to ESRD, but AER was still a better predictor of progression in this late stage of DN.

Another result of our study is that in the microalbuminuria group, before the adjustment with AER, L-FABP was an independent predictor of progression to macroalbuminuria (HR 1.40 [95% CI 1.10–1.79], P = 0.006), and after adjustment for AER, there was surprisingly a protective HR of 0.67 (0.47–0.95, P = 0.02). This result may be a consequence of lower statistical power in this group (46 progressors) or a stronger correlation between AER and L-FABP (r = 0.49) in patients with microalbuminuria, although these alternatives would not explain why L-FABP was an independent predictor in the first place. Another possible explanation could be an effect of medication, because L-FABP was no longer significant in the microalbuminuria group after adjustment for ACE inhibitors or any antihypertensive medication. This is no surprise, because treatment with ACE inhibitors strongly reduces the AER and/or L-FABP levels and influences progression of DN. The lower HR may also be the consequence of a possible protective role of L-FABP against tubulointerstitial damage aggravated by elevated AER, but we cannot prove this possible hypothesis (24).

Our results regarding prediction of DN progression are due to the continuous increase in the L-FABP levels alongside the worsening of the nephropathy stage (10,16). The pathophysiological role of this continuous increase is not completely known but may mirror different mechanisms across DN stages. In early diabetes, before the onset of microalbuminuria, mild hyperglycemia and activation of the intrarenal renin-angiotensin-aldosterone system (RAAS) may lead to oxidative stress at the postglomerular capillary level (25,26). This in turn decreases the availability of NO, which, together with RAAS activation and functional denervation, may lead to vasoconstriction and hypoxia in the tubular cells (27,28). Chronic hypoxia might then trigger *L-FABP* gene overexpression and an increased urinary excretion of L-FABP (29). That an early increase in L-FABP might be independent of AER is further supported by the poor correlation between the two variables (r = 0.15) in the normoalbuminuric patients as well as the independent predictive value of L-FABP for the progression from normal AER to microalbuminuria. In addition, L-FABP increase seems to be connected with tubular injury rather than diabetes itself because L-FABP was poorly correlated with HbA_{1c} (r = 0.06 in nondiabetic subjects; r = 0.11 in patients with type 1 diabetes and normal AER). Once microalbuminuria appears, binding of fatty acids to albumin may trigger fatty acid overload in the proximal tubules, and the *L-FABP* gene may, as a consequence, be upregulated to increase

the free fatty acid transport into the mitochondria. The urinary excretion of L-FABP may then increase again, but such a mechanism has still been considered controversial (8,30,31). At the late stages, oxidative stress and hypoxia (accentuated by anemia) probably cooperate with the elevated AER and cause an L-FABP elevation (28).

The strengths of this study are the large number of patients, long follow-up data of patients, and thorough phenotypic characterization. One potential limitation of the study is that we have no data regarding anemia. Anemia may already be present at the early stages of DN and can potentially increase urinary L-FABP if it is severe enough (32,33). However, at least severe anemia was not an issue in this study because none of the patients received erythropoietin or other treatment for anemia.

In summary, this study shows that L-FABP is an independent predictor of DN progression, irrespective of the disease stage. L-FABP used alone or together with AER may not improve the risk prediction of DN progression in patients with type 1 diabetes, but further studies are needed in this regard.

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N.M.P. researched data, performed statistical analyses, and wrote the manuscript. C.F., M.S., L.T., and A.B. researched data, contributed to discussion, and reviewed and edited the manuscript. P.M.H., and P.-H.G. contributed to discussion and reviewed and edited the manuscript. P.-H.G. is the guarantor of this study 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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