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Association between changes in the mRNA expression of platelet activating factor receptor in peripheral blood mononuclear cells and progression of diabetic nephropathy

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Abstract

Introduction: Several studies have recently pointed out the role of many inflammatory mediators in the progression of diabetes complications. We had previously demonstrated that mRNA expression of platelet activating factor receptor (PAFR) in peripheral blood mononuclear cells (PBMCs) was associated with urinary albumin to creatinine ratio (ACR) and forearm flow-mediated dilatation in patients with type 2 diabetes. In an attempt to elucidate this association, patients were followed up for 1 year.

Materials and Methods: We recruited 95 patients from the hospital outpatient clinic, among whom 86 were followed up for 1 year (normoalbuminuria: 40 patients, microalbuminuria: 25 patients, macroalbuminuria: 21 patients). We then measured their baseline and 12 month characteristics and collected blood samples to extract PBMCs and measure gene expressions.

Results: Despite higher mRNA expression of PAFR in PBMCs among patients with macroalbuminuria, the rise in its value was not associated with biomarkers of nephropathy, while baseline values were not associated with progression of nephropathy. Moreover, changes in mRNA expression of PAFR were correlated with changes in ACR in all patients ($r = 0.225$, $p = 0.037$) and estimated glomerular filtration rate in patients with macroalbuminuria ($r = -0.438$, $p = 0.047$) during the follow-up period.

Conclusion: Our findings indicate that even though no causal relationship exists between diabetic nephropathy and elevated expression of PAFR in PBMCs, their close association; signifies the presence of another common mechanism that could induce both events. Given these findings, the PAF/PAFR interaction could clarify corresponding mechanisms involved in diabetic complications.

Key words: Diabetic Nephropathy, Follow-up Study, Macrovascular Complication, Inflammation

Introduction

Over the last few decades, the prevalence of cardiovascular disease (CVD), chronic kidney disease (CKD), and diabetes, leading causes of death globally and regionally [1], has increased considerably [2], while interaction between these conditions have become the focus of extensive research. A multitude of data have already indicated CKD as an independent predictor of myocardial infarction, stroke, and death [3] and microalbuminuria as a risk marker for cardiovascular mortality among subjects with and without diabetes [4].

A study in the UK found CVD to be the most common cause of death at all stages of nephropathy with an increasing trend for higher disease stages [2]. Another study in the United States found a 63% CVD prevalence in patients with CKD and a 5.8% CVD prevalence in those without the same [5], indicating a direct correlation between CVD prevalence and severity of CKD [5,6].

In an attempt to explain the underlying mechanisms, we had previously conducted a cross-sectional study [7] on the assumption that any molecular change in peripheral blood mononuclear cells (PBMCs) throughout the process of diabetes may cause renal dysfunction and cardiovascular disease through impaired endothelial function [8]. Out of all studied genes associated with inflammation, reactive oxygen species (ROS) genesis, and cell adherence, the link between mRNA expression of platelet activating factor receptor (PAFR) in PBMCs of patients with diabetes and the level of albuminuria and forearm flow mediated dilatation (FMD; positive and negative association, respectively) had been identified as the strongest. Moreover, the prevalence of CVD and cerebrovascular diseases was significantly higher in patients with higher mRNA expression of PAFR in PBMCs [7].

Our study had been the first to demonstrate that mRNA expression of PAFR in PBMCs was correlated with ACR and FMD. However, the causal relationship between the two remains to be described. Moreover, we were unable to assess the influence of glycemic control, hypertension, or dyslipidemia on mRNA expression of PAFR in PBMCs. Based on these findings, the significance of the causal relationship between diabetic nephropathy and mRNA expression of PAFR in PBMCs becomes paramount. Accordingly, we need to determine whether PAFR acts as the cause of albuminuria and subsequently decreases estimated glomerular filtration rate (eGFR), impairs endothelial function and FMD, and eventually causes vascular

events or whether the reverse is true. This follow-up study was designed to further elucidate the role mRNA expression of PAFR in PBMCs plays in the development of diabetic nephropathy.

Materials and Methods

This follow-up study was designed to evaluate the relationship between mRNA expression of PAFR in PBMCs and nephropathic changes in patients with diabetes. As detailed in our previous study, patients with type 2 diabetes who visited the diabetes clinic at Kagoshima University Hospital were recruited excluding those with allergic diseases, connective tissue diseases, hepatitis C or hepatitis B infections, other glomerulonephritis, hematuria, malignant diseases in the past three years, ongoing steroid or immunosuppressive therapy, kidney transplantation, or nephrectomy [7]. All patients with macroalbuminuria were recruited (27 patients). Patients with normo- or microalbuminuria were subsequently recruited to obtain groups matched for age, gender, body mass index (BMI), and HbA1c. Ultimately, 95 patients were recruited into the study and classified according to the three clinical stages of diabetic nephropathy based on measured values of the ACR: normoalbuminuria, ACR < 30 mg/g Cr (n = 42); microalbuminuria, 30 ≤ ACR < 300 mg/g Cr (n = 28); macroalbuminuria, ACR ≥ 300 mg/g Cr (n = 25). Among the included patients, 86 were followed for 1 year (average of 12 months) between July 2014 and October 2016. The study was approved by the Kagoshima University Hospital Ethics Committee (#26-32 dated 2014.6.30, #26-179 dated 2015.2.20, #28-255 dated 2017.3.6), and all participants provided written informed consents before participation.

We collected fasting blood samples at 0 months (hereafter termed “baseline”) and 12 months (12M), as well as two early morning urine samples for two consecutive days on both occasions. Physical examination including anthropometric measures and blood pressure were also measured during the first visit.

Blood and urine data analyses

Urine samples were sent to SLR Inc. (Kagoshima, Japan) for ACR measurement. Blood samples collected in NaF-coated tubes were sent for glucose and HbA1c analyses, while those collected in EDTA-coated tubes were used to measure gene expression in PBMCs. We also collected blood samples in serum-separating tubes to analyze LDL-cholesterol (LDL-C), HDL

cholesterol (HDL-C), triglycerides (TG), and creatinine (Cr). Glucose, HbA1c, LDL-C, HDL-C, TG, and Cr were immediately measured at the Kagoshima University Hospital clinical laboratory center. Estimated GFR was calculated based on serum Cr, age, and gender [eGFR = 194 × (Cr) − 1.094 × (age) − 0.287 × (0.739 if female)].

PBMC preparation and RNA extraction

We twice diluted 10 ml of blood collected in EDTA-coated tubes with phosphate-buffered saline (PBS) and then layered it over 10 ml of Lymphopreb™ (Axis-Shield, Oslo, Norway) in a centrifuge tube. The sample was then centrifuged at 800 g for 20 minutes at room temperature (RT). Afterwards, the sample–medium interface containing mononuclear cells was transferred into a new centrifuge tube and rinsed with three volumes of PBS. The mononuclear cell pellet at the bottom of the tube was then collected after centrifugation at 1500 g for 10 minutes.

To extract RNA, the cell pellet was then homogenized with 0.75 ml of TRIzol® reagents (Life Technologies) and incubated for 5 minutes at RT. Thereafter, 0.2 ml of chloroform was added to the homogenate. After another 10 minutes of incubation at RT, the homogenate was centrifuged at 12000 g for 15 minutes at 4 °C in a micro-centrifuge (Eppendorf 5471R). Afterwards, the aqueous phase containing RNA was carefully transferred into a new micro-centrifuge tube. Adding 0.5 ml of 100% isopropanol and centrifuging at 12000 g for 10 minutes at 4 °C precipitated the RNA, and the RNA pellet was washed with 1 ml of 75% ethanol and centrifuged one last time at 7500 g for 5 minutes at 4 °C. After aspirating the supernatant, the pellet was air dried for 5 minutes at RT and dissolved in 100 µl of RNAase-free water. RNA purity and quantity was measured spectrophotometrically using NanoDrop 1000 (Thermo Fisher Scientific, USA). All samples had an A260/A280 ratio of 1.70–2.00 indicating that the RNA was highly purified.

Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Real-time quantitative RT-PCR was performed to determine the relative expression levels of mRNA. First, single-stranded cDNA was synthesized from RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Accordingly, 1 µg of total RNA was used to make 20 µl of reaction reagents, which was then incubated at 37 °C for 2 hours followed by denaturation at 85 °C for 5 minutes using Power BLOCK (ATTO, Japan). Afterwards, quantitative RT-PCR was performed using a StepOnePlus thermocycler (Applied Biosystems)

according to the manufacturer's protocol. The reaction included adding 100 ng of cDNA to a total of 20 µl of PCR reaction mix containing TaqMan qPCR master mix, TaqMan gene expression assays (Applied Biosystem), and RNAase-free water. The reactions were performed in duplicates on 96-well plates under the following conditions: holding stage of 20 seconds at 95 °C, followed by 40 cycles consisting of 1 second at 95 °C and 20 s at 60 °C. Hypoxanthine phosphoribosyltransferase-1 (HPRT1) was quantified as a housekeeping gene. Based on previous research, we selected a particular set comprising the following genes related to the pathology of diabetic nephropathy or atherosclerosis for analysis: PAFR, cluster differentiation 36 molecule (CD36), IFN-gamma-inducible protein 10 (CXCL10, IP-10), and tissue inhibitor of metalloproteinase 2 (TIMP2). The arbitrary unit was determined by the ratio of mRNA expression of the target gene to that of HPRT1.

Statistical analysis

Data are expressed as mean ± standard deviation (SD), median (interquartile range; IQR), or numbers. Normally distributed variables between groups were compared using one-way analysis of variance (ANOVA; followed by Tukey or Games-Howell post hoc test), while paired t-tests were used to compare baseline and 12-month (12M) values. For skewed characteristic variables, log-transformed data were used. Gene mRNA expressions in PBMCs were analyzed using the Kruskal-Wallis test followed by pairwise comparison, while the Wilcoxon signed-rank test was used to compare baseline and 12-month values. Qualitative variables were analyzed using the Chi-square test.

Correlations between any two variables were determined using Pearson or Spearman rank–order correlation tests. For skewed variables, log-transformed data were used. All statistical analyses were performed using SPSS version 22.0 (SPSS Inc, USA) with *p* values less than 5% being considered statistically significant.

Results

Characteristics of patients before and after the follow-up period based on nephropathy stage

To study the effects of nephropathy on mRNA expression of PAFR in PBMCs, 86 patients with type 2 diabetes were followed up for 1 year. Table 1 shows the patient characteristics based on nephropathy stage. Groups were matched for age and gender. Although baseline and 12M levels of BMI, FPG, HbA1c, and triglyceride did not differ among the groups, significant differences in baseline and 12M levels of HDL -cholesterol were observed ($p < 0.05$). As expected, significant differences in both eGFR and ACR levels were found between the groups (at both baseline and 12M, $p < 0.001$); however, a noticeable decrease in patients with macroalbuminuria was observed during the follow-up. Although mRNA expression of PAFR was significantly higher in those with macroalbuminuria ($p < 0.005$) at both baseline and the end of the trial, it did not change significantly during the follow-up, suggesting that the increase in the mRNA expression of PAFR in PBMCs during this time was not associated with nephropathy at either baseline or after the follow-up. However, this was not the case for patients with microalbuminuria or normoalbuminuria who showed a discernible rise during this time ($p < 0.005$). This suggests that advanced nephropathy state did not induce mRNA expression of PAFR in PBMCs.

Baseline factors affecting changes in mRNA expression of PAFR in PBMCs

Given the significant increase in the mRNA expression of PAFR in PBMCs during the follow-up period, factors that could be associated with this increase other than nephropathy stage were analyzed. We found that none of the usual biomarkers i.e., glycemic factors, systolic blood pressure, BMI, or lipid profile at baseline, predicted the increase in mRNA expression of PAFR in PBMCs (Table 2). Furthermore, the association between elevated mRNA expression of PAFR and treatment regimens during the follow-up period was investigated. As shown in supplementary Table 1, the patients were treated with several kinds of antihyperglycemic, antihyperlipidemic, or antihypertensive drugs, none of which had shown any effect on changes in mRNA expression of PAFR.

Factors associated with changes in mRNA expression of PAFR in PBMCs during the study

Figure 1 shows a significantly positive correlation between changes in mRNA expression of PAFR in PBMCs and changes in urinary ACR during the follow-up period in all patients (r = 0.225, p = 0.037). The multivariate analysis shows that the correlation among the changes in urinary ACR and those in mRNA expression of PAFR in PBMCs are independent to known cofounding factors such as systolic BP, obesity, and usage of ARB or ACEI (Table 3). On the other hand, changes in eGFR (as presented in Figure 2) showed a negative correlation with changes in mRNA expression of PAFR in PBMCs only in patients with macroalbuminuria (r = −0.438, p = 0.047). However, baseline mRNA expression of PAFR in PBMCs did not predict progression of diabetic nephropathy [rise in ACR (r = −0.192, p = 0.076) or deterioration of eGFR (r = −0.177, p = 0.102), Figure 3]. This again suggests that mRNA expression of PAFR in PBMCs did not cause diabetic nephropathy but rather was associated with its progression.

We also found strong positive correlations between changes in mRNA expression of PAFR in PBMCs and changes in mRNA expression of other genes associated with development of atherosclerosis, namely CD36 (r_s = 0.489, p = 0.000), IP10 (r_s = 0.337, p = 0.001), and TIMP2 (r_s = 0.660, p = 0.000; Figure 4).

Discussion

Similar to our previous report [7], the present study showed that mRNA expression of PAFR in PBMCs was significantly higher in patients with advanced nephropathy both at baseline and after 12 months of follow-up. However, the increase in its expression during this period was not associated with biomarkers of nephropathy either at baseline or after the follow-up. Furthermore, we observed that the mRNA expression of PAFR in PBMCs at baseline was not associated with progression of nephropathy. These observations indicate no causal relationship between diabetic nephropathy and elevated mRNA expression of PAFR in PBMCs. This close association, however, indicates the presence of another common mechanism such as inflammation that induces both events, nephropathy progression and elevated mRNA expression of PAFR in PBMCs. To support this idea, we found a weak albeit significant correlation between changes in mRNA expression of PAFR in PBMCs and changes in urinary ACR in all patients or eGFR in patients with macroalbuminuria during the follow-up period.

Various risk factors involved in the progression of diabetic nephropathy [1,9-11], such as poor glycemic control and elevation of HbA1c level [12,13], hypertension [14,15], obesity [16], and dyslipidemia [17], have been well investigated. On the other hand, some interventions that utilize renin angiotensin system (RAS) inhibitors [18] like angiotensin receptor blockers (ARB) [19] and angiotensin-converting enzyme inhibitor (ACEI) [20], sodium glucose co-transporter-2 (SGLT-2) inhibitors [21], glucagon-like peptide-1 (GLP-1) receptor agonists [22], statins [23], and fibrates [24] have been shown to delay the progression of nephropathy. Therefore, we analyzed the effects of these known factors on changes in mRNA expression of PAFR in PBMCs during the follow-up period. Accordingly, we found that none of the aforementioned factors were associated with changes in mRNA expression of PAFR in PBMCs during the follow-up period. However, changes in mRNA expression of PAFR in PBMCs were associated with the changes in mRNA expressions of other genes, namely CD36 [25,26], IP10 [27], and TIMP2 [28,29] in PBMCs. These genes, as well as PAFR, have been known to be regulated by mechanisms related to inflammatory processes [25,28], and similar to existing literature, we show that they are upregulated in diabetic nephropathy [26,27,29]. Considering that glomerular inflammation is an important factor in the progression of diabetic nephropathy [30,31], we can surmise that a common factor related thereto may be involved in the progression of nephropathy and upregulation of mRNA expression of PAFR in PBMCs. Further study is definitely needed to identify this common factors as inflammation.

Our findings of associations between changes in mRNA expression of PAFR in PBMCs and changes in urinary ACR or eGFR may prove to be clinically important. The mRNA expression of PAFR in PBMCs could easily be used as an indicator of the progression of nephropathy. Although the measurements of urinary ACR or eGFR have already been established as biological markers of diabetic nephropathy, the expression of PAFR in PBMCs might add some crucial details, such as important information regarding the glomerular microenvironment. Furthermore, considering the role of PAFR in PBMCs on the development of atherosclerosis, the elevated expression of PAFR in patients with diabetic nephropathy might explain the link between the development of diabetic nephropathy and the increased risk of cardiovascular events [32]. In fact, as mentioned in our previous paper, patients with a history of vascular events showed higher levels of PAFR expression in PBMCs [7]. Future studies will reveal the

significance of elevated mRNA expression of PAFR in PBMCs in the prevention or even treatment of vascular lesions in patients with diabetic nephropathy.

Given the nature of the present study, some methodological limitations should be considered. Due to the lack of healthy controls and the small number of participants with macroalbuminuria, analyzing data relevant to more advanced stages of nephropathy and defining a causal relationship between mRNA expression of PAFR and diabetic nephropathy were inconclusive. Furthermore, the short period of follow-up did not allow us to witness any significant changes in albuminuria and/or the state of nephropathy. Most importantly, given the nature of PAF and PAFR, i.e., dose-, regional-, and species-dependent nature of their effects [33,34], explaining molecular mechanisms without more controlled cell culture studies proved to be insufficient. Notwithstanding, our data may actually explain why only a fraction of patients with diabetes suffer from nephropathy and other macrovascular complications of diabetes at different progression rates [11]. This offers new therapeutic opportunities, such as using PAFR antagonists to treat patients with diabetic nephropathy who have higher mRNA expression of PAFR, especially those with microalbuminuria, to prevent disease progression and renal failure. Moreover, PBMCs are easy to collect and may provide a more accessible specimen for gene expression investigations.

In conclusion, changes in mRNA expression of PAFR in PBMCs were associated with progression of nephropathy in patients with either microalbuminuria or macroalbuminuria. Given the interactions between PAF expressed on endothelial cell surface and PAFR expressed on PBMCs surface and the association between endothelial dysfunction and elevated expression of PAFR in PBMCs, the PAF/PAFR interaction might shed some light on diabetic complications and the corresponding mechanisms. Thus, measuring mRNA expression of PAFR in PBMCs might prove useful as a screening test to focus nephroprotective treatment to those at higher risk.

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Disclosure

The authors declare no conflict of interest associated with this manuscript.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consents were obtained from all participants for being included in the study.

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Figure Legends

Fig. 1 Correlation between changes in the mRNA expression of PAFR in PBMCs and changes in urinary ACR during the follow-up period in all patients

ΔPAFR (arbitrary unit) was calculated for changes in the mRNA expression of PAFR in PBMCs during the follow-up period (12M value − baseline value)

ΔACR (mg/g.Cr) was calculated for changes in urinary ACR during the follow-up period (12M value − baseline value)

Log-transformed values were used

Fig. 2 Correlation between changes in the mRNA expression of PAFR in PBMCs and changes in eGFR during the follow-up period in all patients (a) and in those with macroalbuminuria (b)

ΔPAFR (arbitrary unit) was calculated for changes in the mRNA expression of PAFR in PBMCs during the follow-up period (12M value − baseline value) *Log-transformed values were used

ΔeGFR (ml/min/1.73m^2) was calculated for changes in eGFR during the follow-up period (12M value − baseline value)

Fig. 3 Correlation between the mRNA expression of PAFR in PBMCs at baseline and change in urinary ACR (a) or eGFR (b) during the follow-up period in all patients

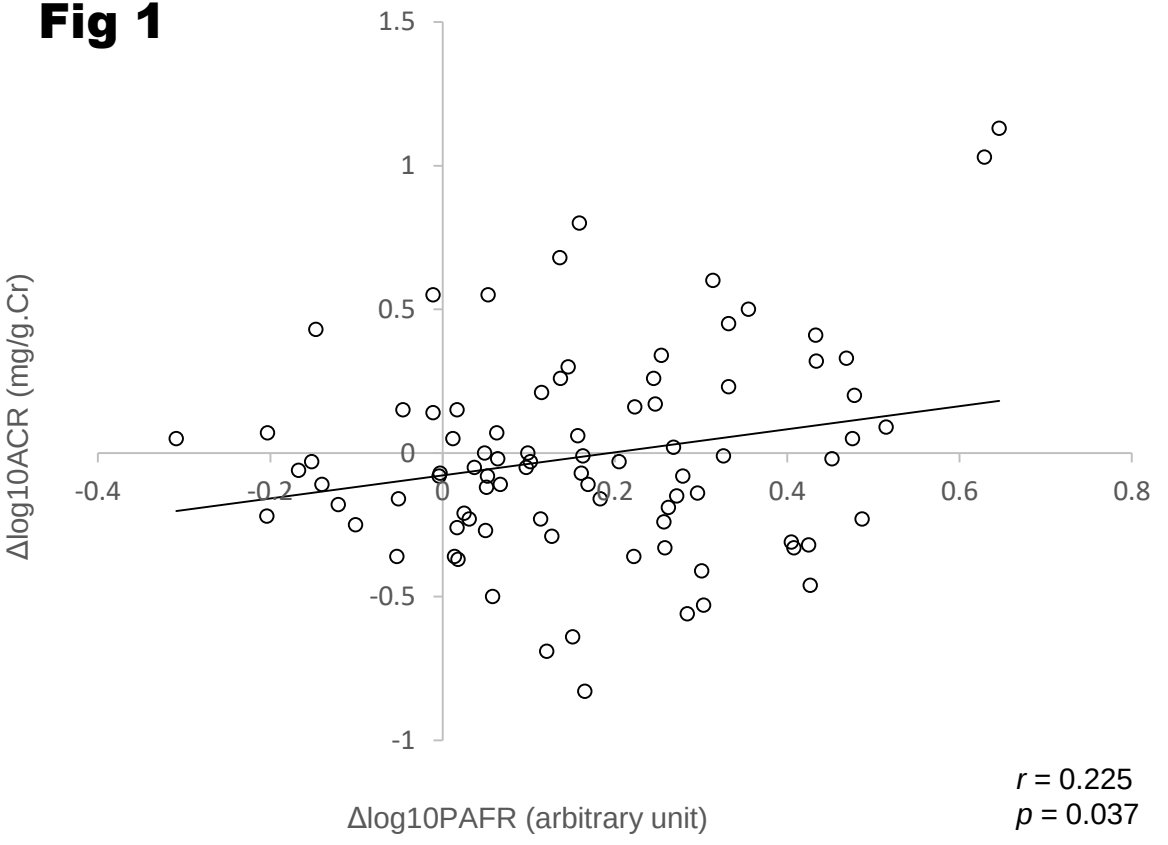
ΔACR (mg/g.Cr) was calculated for changes in urinary ACR during the follow-up period (12M value − baseline value) *Log-transformed values were used

ΔeGFR (ml/min/1.73m^2) was calculated for changes in eGFR during the follow-up period (12M value − baseline value)

Fig. 4 Correlation between changes in the mRNA expression of PAFR and other genes [CD36 (a), IP10 (b), and TIMP2 (c)] in PBMCs during the follow-up period in all patients

Δgene (arbitrary unit) was calculated for changes in the mRNA expression of PAFR, CD36, IP10, and TIMP2 in PBMCs during the follow-up period (12M value − baseline value)
*Spearman's rank test results are reported as ρ

Fig 1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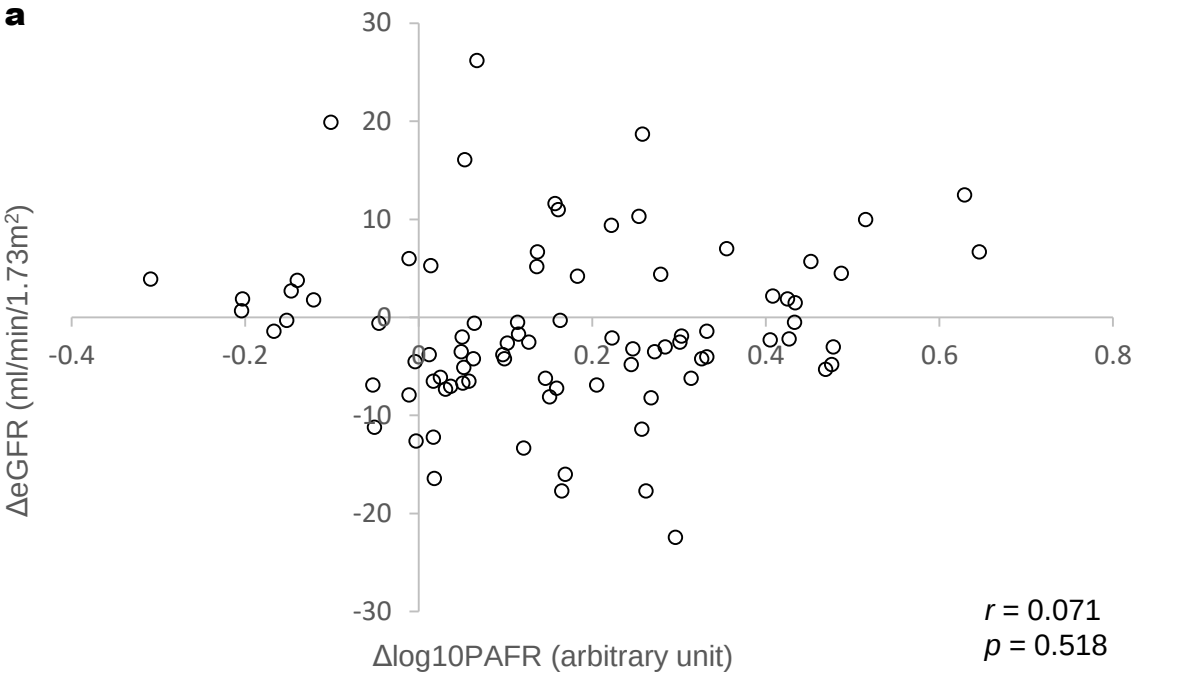
r = 0.225
p = 0.037

Fig 2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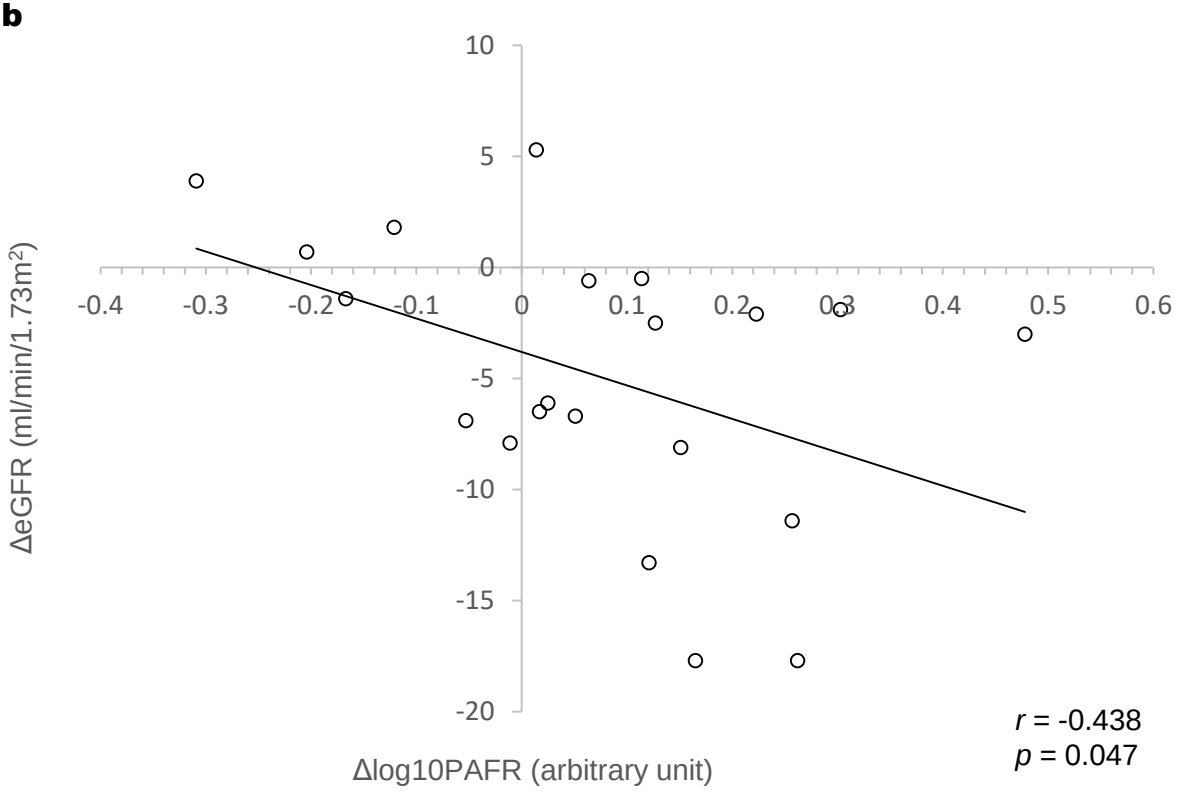
p = 0.518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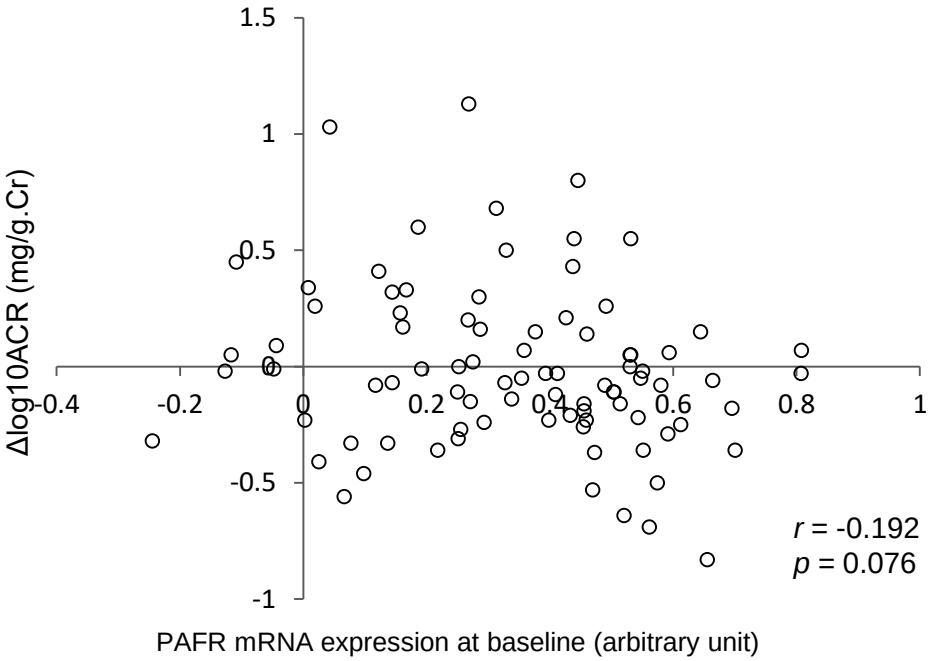
ΔeGFR (ml/min/1.73m²)
Δlog10PAFR (arbitrary unit)
r = -0.438
p = 0.047

Fig 3

a


1.5
1
0.5
0
-0.5
-1
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Δlog10ACR (mg/g.Cr)
r = -0.192
p = 0.076
PAFR mRNA expression at baseline (arbitrary unit)

b

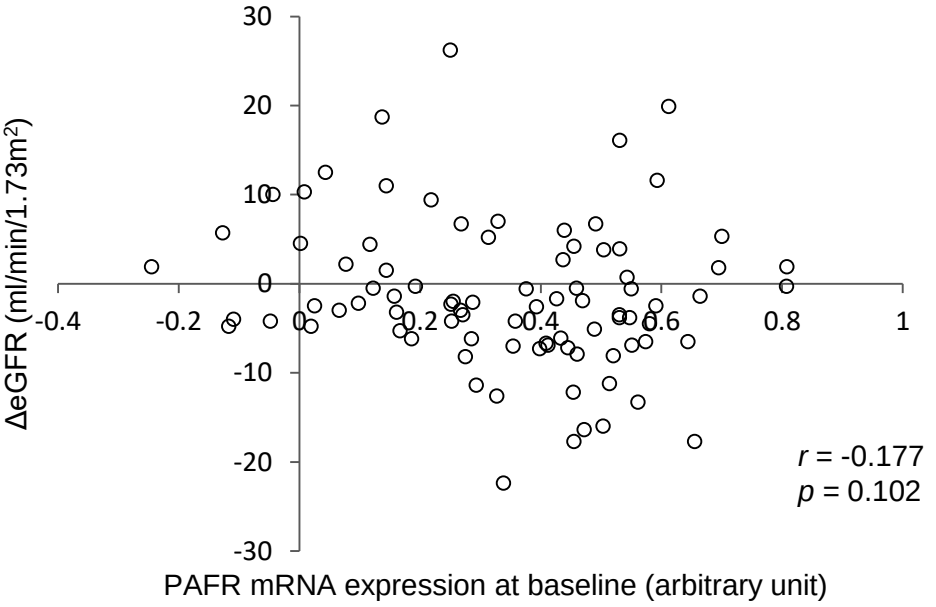

30
20
10
0
-10
-20
-30
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
ΔeGFR (ml/min/1.73m²)
r = -0.177
p = 0.102
PAFR mRNA expression at baseline (arbitrary unit)

Fig 4

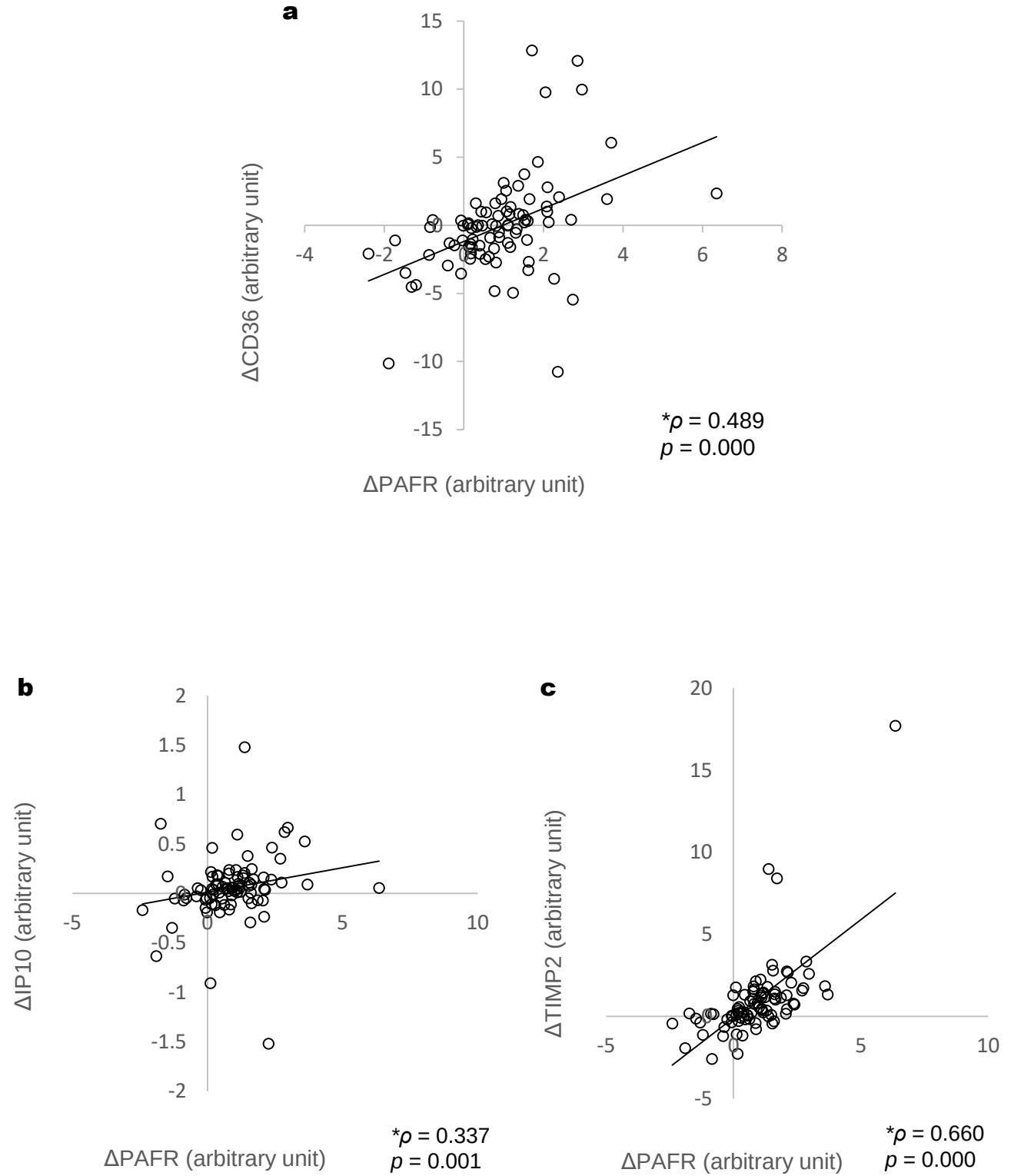

a
15
10
5
0
-5
-10
-15
-4
-2
0
2
4
6
8
ΔCD36 (arbitrary unit)
ΔPAFR (arbitrary unit)
*ρ = 0.489
p = 0.000
b
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-5
0
5
10
ΔIP10 (arbitrary unit)
ΔPAFR (arbitrary unit)
*ρ = 0.337
p = 0.001
c
20
15
10
5
0
-5
-5
0
5
10
ΔTIMP2 (arbitrary unit)
ΔPAFR (arbitrary unit)
*ρ = 0.660
p = 0.000

Table 1. Characteristics of the patients based on nephropathy stage

variable	**Base line**				**12 month**			
	normo n=40	micro- n=25	macro- n=21	*p*	normo n=40	micro- n=25	macro- n=21	*P*
Age (years)	64.3 ± 9.4	61.5 ± 11.2	64.4 ± 12.6	0.537				
Gender (M/F)	27/13	18/7	14/7	0.908				
BMI (kg/m^2)	25.1 ± 4.1	26.6 ± 5.76	24.8 ± 5.2	0.378	24.7 ± 4§	26.3 ± 5.46§	24.9 ± 5.34	0.417
FBG (mg/dl)	141 ± 28	148.4 ± 41.4	131.5 ± 29.2	0.208	134.8 ± 26	145 ± 41	157.5 ± 55.6§	0.165
HbA1c (%)	7 ± 0.6	7.2 ± 1.2	6.9 ± 1	0.614	7 ± 0.7	7.2 ± 1.2	6.9 ± 1	0.410
TG (mg/dl)	125.4 ± 70.6	171.8 ± 104	118.6 ± 52	0.050	112 ± 59	215 ± 296*	131 ± 70	0.057
L-DLC (mg/dl)	110.8 ± 27.7	106.3 ± 34.8	98 ± 26.4	0.300	104.8 ± 27.9	100.6 ± 22.5	96 ± 27.5	0.464
H-DLC (mg/dl)	59.5 ± 13.9	49.8 ± 11.9*	63.6 ± 19.7‡	0.009	59.5 ± 13.4	49.9 ± 11.8*	59.3 ± 16.8	0.011
eGFR (ml/min/1.73m^2)	70.7 ± 17	67.6 ± 27	42.2 ± 22.7*‡	0.000	69.7 ± 16.1	68.7 ± 28.2	37 ± 22*‡§	0.000
ACR (mg/g.Cr)	11.6 ± 7.9	123.4 ± 85.6*	1311 ± 1115*‡	0.000	15.5 ± 14.5	169 ± 144*	906 ± 910*‡§	0.000
PAFR[a] (arbitrary unit)	1.7(1.2-2.8)	2.4(1.8-3.3)	3.4(2.8-4.2)* ‡	0.000	3(2.3-3.8)§	3.2(2.2-4.2)§	3.8(3-5.2)*	0.023

Data are presented as mean ± SD. [a] mRNA expression of PAFR in PBMCs is presented as median (IQR).

p values were calculated using ANOVA followed by post-hoc Tukey or Games-Howell post hoc test (for skewed variables, log-transformed data were used).

* $p<0.05$ vs. normoalbumirics ‡$p<0.05$ vs. microalbuminurics

BMI: Body Mass Index; FBG: Fasting Blood Glucose level; TG: Triglyceride level; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein Cholesterol; eGFR: estimated Glomerular Filtration Rate [$eGFR= 194 \times (Cr)^{-1.094} \times (age)^{-0.287} \times (0.739$ if female)]; ACR: Albumin to Creatinine Ratio; PAFR: Platelet Activating Factor Receptor.
Differences between baseline and 12-month values were calculated by paired t-test (for skewed variables, log-transformed data were used). § $p<0.05$ vs. baseline

Table 2. Associations between baseline patient characteristics and changes in mRNA expression of PAFR in PBMCs

variable	**mean ± SD**	**Correlations with**					
		baseline PAFR		12-M PAFR		Changes in PAFR	
		ρ	*p value*	*ρ*	*p value*	*ρ*	*p value*
Age (years)	63.5 ± 10.7	0.068	0.532	0.026	0.810	-0.036	0.743
BMI (kg/m^2)	25.5 ± 4.9	-0.049	0.651	-0.036	0.745	-0.033	0.760
SBP (mmHg)	131.8 ± 16.9	0.154	0.158	0.133	0.224	-0.018	0.873
FBG (mg/dl)	140.8 ± 32.9	0.016	0.884	-0.060	0.582	-0.032	0.772
HbA1c (%)	7 ± 0.9	-0.106	0.332	-0.096	0.379	0.049	0.652
TG (mg/dl)	137.2 ± 80.6	-0.034	0.758	-0.043	0.695	-0.074	0.498
L-DLC (mg/dl)	106.3 ± 29.7	-0.098	0.369	-0.188	0.084	-0.123	0.259
H-DLC (mg/dl)	57.7 ± 15.8	0.103	0.346	0.013	0.907	-0.067	0.541
FMD (%)	4.6 ± 2.5	-0.370	0.001*	-0.231	0.036*	0.160	0.149

Data are presented as mean ± SD. Correlations were calculated using spearman's rank correlation. * $p<0.05$ was considered as significant.

BMI: Body Mass Index; SBP: Systolic Blood Pressure; FBG: Fasting Blood Glucose level; TG: Triglyceride level; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein Cholesterol; FMD: Flow Mediated Dilation; PAFR: Platelet Activating Factor Receptor.

Table 3. Univariate and Multivariate analysis of renal dysfunction risk factors as independent predictors for change in urinary ACR.

	Δlog10ACR	
Predictors	Univariate model	Multivariate model
Δlog10PAFR	0.255*	0.221*
SBP		-0.241*
log10BMI		0.247*
Usage of ARB/ACEI		0.115
r^2	0.051*	0.146*

The table gives standard regression (β values). *$p < 0.05$. For skewed variables, log-transformed data were used.
ACR: Albumin to Creatinine Ratio; PAFR: Platelet Activating Factor Receptor; SBP: Systolic Blood Pressure; BMI: Body Mass Index; ARB/ACEI: Angiotensin Receptor Blocker/ Angiotensin-Converting Enzyme Inhibitor.