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### **► To cite this version:**

Vincent Dupont, Constance Xhaard, Isabelle Behm-Ansmant, Emmanuel Bresso, Quentin Thuillier, et al.. Multiomomic profiling of new-onset kidney function decline: insights from the STANISLAS study cohort with a 20-year follow-up. *Clinical Kidney Journal*, 2024, 17 (8), pp.sfae224. 10.1093/ckj/sfae224 . hal-04660216

**HAL Id: hal-04660216**

**<https://hal.univ-lorraine.fr/hal-04660216>**

Submitted on 29 Aug 2024

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ORIGINAL ARTICLE

# Multiomic profiling of new-onset kidney function decline: insights from the STANISLAS study cohort with a 20-year follow-up

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## ABSTRACT

**Background.** Identifying the biomarkers associated with new-onset glomerular filtration rate (GFR) decrease in an initially healthy population could offer a better understanding of kidney function decline and help improving patient management.

**Methods.** Here we described the proteomic and transcriptomic footprints associated with new-onset kidney function decline in an initially healthy and well-characterized population with a 20-year follow-up. This study was based on 1087 individuals from the familial longitudinal Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux (STANISLAS) cohort who attended both visit 1 (from 1993 to 1995) and visit 4 (from 2011 to 2016). New-onset kidney function decline was approached both in quantitative (GFR slope for each individual) and qualitative (defined as a decrease in GFR of >15 ml/min/1.7 m<sup>2</sup>) ways. We analysed associations of 445 proteins measured both at visit 1 and visit 4 using Olink Proseek<sup>®</sup> panels and 119 765 genes expressions measured at visit 4 with GFR decline. Associations were assessed using multivariable models. The Bonferroni correction was applied.

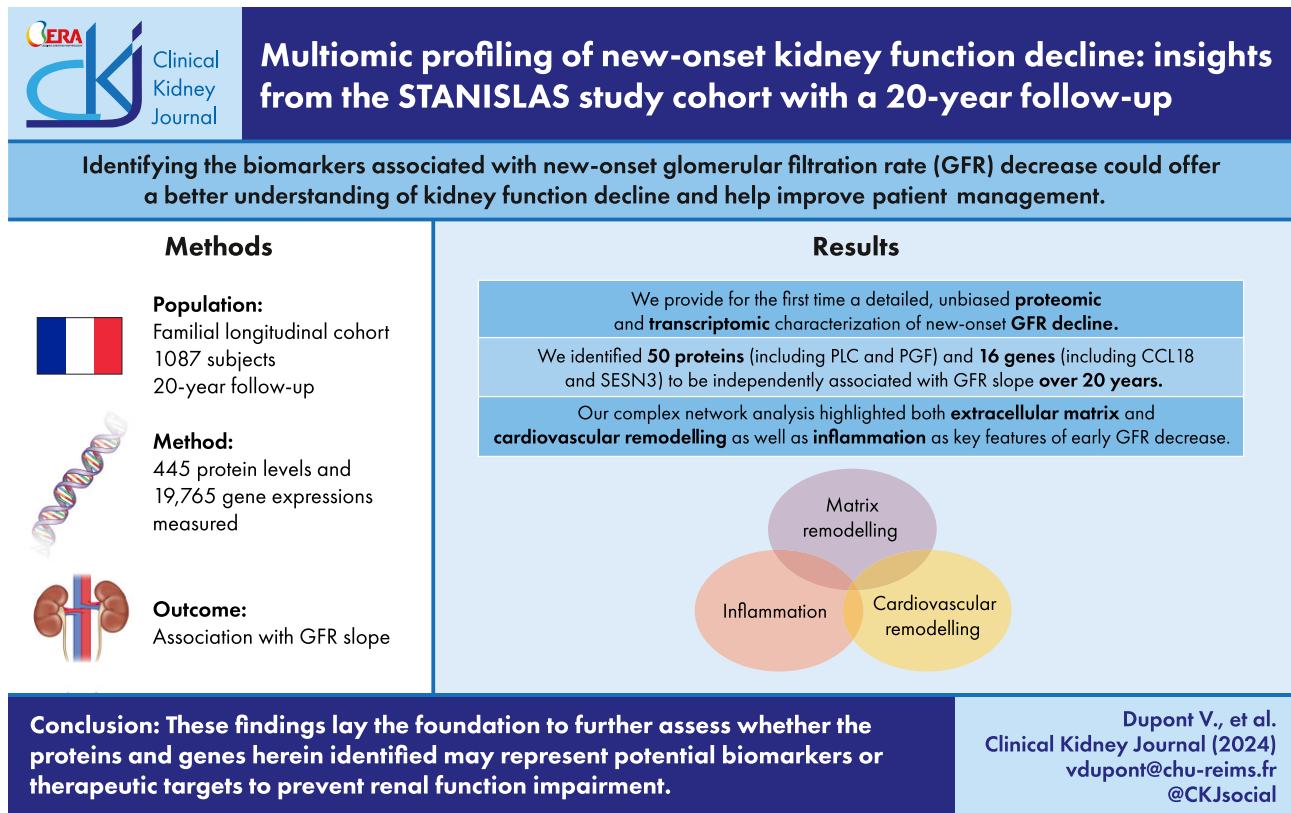
**Results.** We found several proteins (including PLC, placental growth factor (PGF), members of the tumour necrosis factor receptor superfamily), genes (including CCL18, SESN3), and a newly discovered miRNA—mRNA pair (MIR1205—DNAJC6) to be independently associated with new-onset kidney function decline. Complex network analysis highlighted both extracellular matrix and cardiovascular remodelling (since visit 1) as well as inflammation (at visit 4) as key features of early GFR decrease.

Received: 11.4.2024; Editorial decision: 23.6.2024

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**Conclusions.** These findings lay the foundation to further assess whether the proteins and genes herein identified may represent potential biomarkers or therapeutic targets to prevent renal function impairment.

## GRAPHICAL ABSTRACT



**Keywords:** glomerular filtration rate, proteomic, transcriptomic

## KEY LEARNING POINTS

### What was known:

- Multiomic analysis of large cohorts has already allowed to generate multidimensional datasets that improved knowledge on the pathophysiology of multiple diseases.
- Identifying the biomarkers associated with new-onset glomerular filtration rate (GFR) decrease could offer a better understanding of kidney function decline and help improve patient management.

### This study adds:

- Here, we report for the first time the associations between thousands of proteins levels and genes expressions with new-onset kidney function decline over a 20-year period in a large, initially healthy population without comorbidities and using an unbiased multiomic approach.
- The expressions of 50/445 proteins and 16/119765 genes were independently associated with eGFR slope over 20 years.
- Our complex network analysis highlighted both extracellular matrix and cardiovascular remodelling as well as inflammation as key features of early GFR decrease.

### Potential impact:

- Our findings lay the foundation to further assess whether the proteins and genes herein identified may represent potential biomarkers or therapeutic targets of interest to prevent renal function impairment.

## INTRODUCTION

Chronic kidney disease (CKD) represents a global healthcare burden affecting 10% of the worldwide population and is projected to become the fifth leading cause of death by 2040 [1, 2]. Whatever the cause, CKD natural history may lead to end-stage renal disease. Its associated morbidity and mortality, mostly from cardiovascular diseases, increase along with glomerular filtration rate (GFR) decline [3]. Therefore, early diagnosis and management of CKD may offer the potential to substantially reduce the burden from renal function impairment [3, 4]. Hence, identifying potential biomarkers and pathophysiological mechanisms associated with new-onset renal disease could be of major importance, not only for a better understanding of the disease but also for improving the current prevention strategies and treatments [5].

In recent years, high-throughput technologies have considerably revolutionized medical research [6]. Multiomic analysis of large cohorts has already allowed to generate new and multidimensional datasets that improved knowledge on multiple diseases such as cancer, diabetes, and cardiovascular diseases [7–9]. Nowadays, both transcriptomic and proteomic measurements are easily accessible and may represent a promising alternative to better assess early renal function impairment as well [10, 11].

We aimed to describe the transcriptomic and proteomic factors associated with new-onset kidney function decline in an initially healthy, well-characterized population with a 20-year follow-up.

## MATERIALS AND METHODS

### Study cohort

The STANISLAS (Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux) cohort is a single-centre familial prospective cohort of 1006 healthy families (4295 participants) from the Nancy region of France recruited from 1993 to 1995 for visit 1 (V1), then followed each for 5 to 10 years [12]. Visit 4 (V4) took place from 2011 to 2016 and included 1705 participants. Out of 4295 participants, 111/ (2.6%) died between V1 and V4 [12]. Participants and nonparticipants at V4 had similar baseline characteristics [13].

Beyond the clinical, imaging, and routine laboratorial measurements, circulating levels of multiple proteins were assessed at V1 and V4. In addition, a genome-wide association study (GWAS) and a global transcriptome analysis were performed for only the V4 participants. This design allows the assessment of the clinical factors, genetic variants, and transcriptomic and proteomic signatures associated with new-onset pathologies such as early kidney function decline. In the present analysis, we included 1087 participants who attended both V1 and V4 and were at least 18 years old at V1.

The research protocol was conducted according to the Declaration of Helsinki and approved by the local ethics committee (Comité de Protection des Personnes Est III, Nancy, France). All study participants gave written informed consent to participate.

### New-onset kidney function decline definition

GFR decline is causally associated with renal function impairment [14]. Recent data support the use of GFR slope as a primary endpoint for clinical trials focusing on CKD progression [15]. Thus, we chose to approach new-onset kidney function decline in our cohort using two ways: (i) as a quantitative variable, i.e.

the estimated GFR (eGFR) slope between V1 and V4 for each individual, and (ii) as a qualitative variable, i.e. a decrease in eGFR ( $\Delta$ eGFR) of  $>15$  ml/min/1.7 m<sup>2</sup> between V1 and V4 based on the CKD-EPI formula and using plasma creatinine levels [16]. Creatinine measurements were performed using the Jaffe method at V1 and enzymatic method at V4.

### Gene expression and circulating proteins

Baseline (V1) and follow-up (V4) plasma samples were analysed by the TATAA-biocentre using the Olink Proseek® Multiplex cardiovascular II, III, cardiometabolic, organ-damage and inflammatory panels using a proximity extension assay technology [17]. A total of 445 proteins were available for statistical analysis.

Blood DNA was extracted using Genra Puregene Blood Kit (Qiagen, Germany®) and stored at  $-20^{\circ}\text{C}$  only for V4 participants. Genotyping was conducted at the Centre National de Recherche en Génomique Humaine (Evry, France) using the Illumina Global Screening and Exome Arrays [18].

Genes expressions were assessed on a subgroup of 887 individuals having available transcriptomic data. Whole blood RNAs were extracted from PAXgene Blood RNA Tubes (Qiagen, Germany) using MagMAX Isolation Kit (Life Technologies, France) on a KingFisher Duo Prime automated purification system (ThermoFisher Scientific, France). Extracted RNAs were quantified using a Nanodrop spectrophotometer, and their quality was assessed using the RNA ScreenTape system (Agilent, France). Transcriptome analysis was conducted at the Ingénierie Moléculaire et Physiopathologie Articulaire (Vandoeuvre-les-Nancy, France) using Clariom D® assays (Affymetrix, ThermoFisher Scientific, France) [19]. Gene expressions intensities were normalized using 'Limma' [20] (v.3.44.3) implemented in the Transcription Analysis Console Software v.4.0.2 by SST-RMA and then extracted for each individual as normalized intensity values (as  $\log_2$ ) obtained from Clariom D® microarrays.

### Statistical and bioinformatical analysis

For the baseline and follow-up clinical characteristics, continuous variables are expressed as means and standard deviations and compared using Student's t-test or Wilcoxon nonparametric tests. Categorical variables are presented as frequencies and percentages and compared using the Chi-squared test.

The heritability of a phenotype is defined as the proportion of its total variance explained by additive genetic factors. Heritability is optimally estimated in family-based sample populations. Here, we took advantage from the familial structure of our cohort to approach the heritability of eGFR decline. To this aim, we considered both our study population ( $n = 1087$ ) and their children aged  $<18$  years at V1 (total  $n = 1420$ ). We used a linear mixed model that simultaneously included additive genetic effects across the genome, common environment effects shared by the nuclear family and fixed effects. The additive genetic effects were assessed using the Genetic Relatedness Matrix [21]. Estimations were performed using R (v.3.4.1.) and were implemented in the 'gaston' R package (v.1.5.6).

Associations between each of the proteins/genes and eGFR slope between V1 and V4 (ml/min/1.7 m<sup>2</sup>) were assessed using multivariable linear regression models. To strengthen our approach, we also tested the associations between each of the proteins and genes and  $\Delta$ eGFR  $> 15$  ml/min/1.7 m<sup>2</sup> using multivariable logistic regression models. For both approaches, only genes

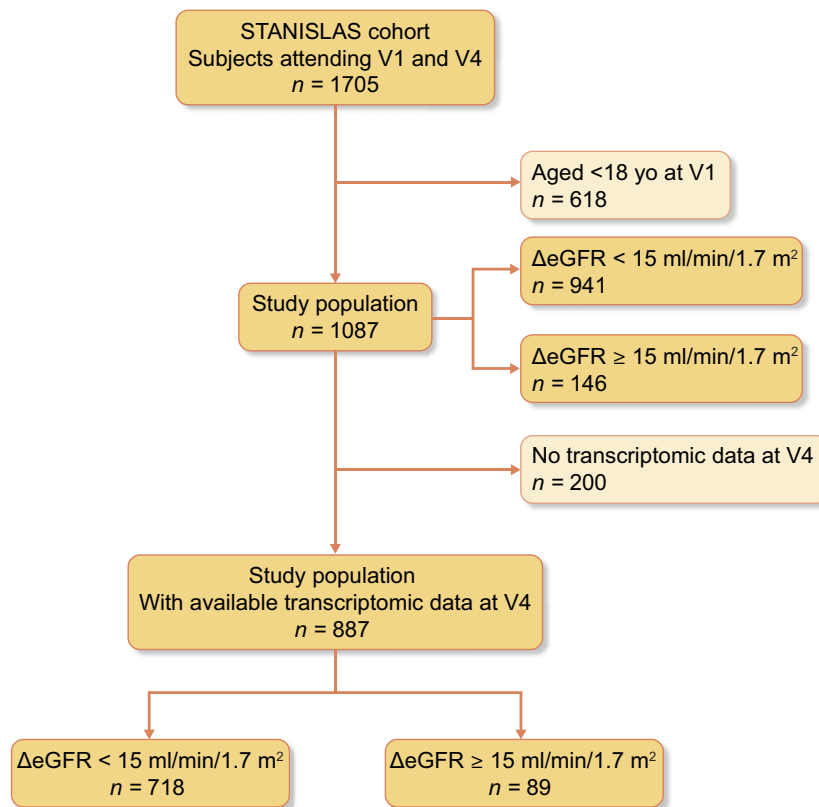


Figure 1: Flow chart of the study.

that were associated with eGFR decline with a false discovery rate  $<10^{-4}$  in univariate analysis were included in multivariate analysis.

The following variables were included in multivariable analysis models: age, sex, body mass index (BMI), current smoker, and eGFR [22]. Baseline eGFR was accounted for in multivariate analysis as a major determinant of eGFR slope as previously suggested [23]. To account for multiplicity, we systematically applied the Bonferroni correction to the *P* values obtained in multivariate analysis [24].

To assess the potential ability of each of the proteins and genes to early identify kidney function decline, we generated receiver operating characteristic (ROC) curves (with 95% confidence interval) for the top ones associated with  $\Delta\text{eGFR} \geq 15 \text{ ml/min/1.7 m}^2$ .

A complex network analysis was performed using the Fight Heart-Failure Graph Knowledge Box (FHF-GKB) [25]. Proteins were imported from UniProt [26]. Pathways and protein-pathway relationships were retrieved from REACTOME [27]. Resulting networks were displayed by Cytoscape (v.3.9.1).

## RESULTS

### Characteristics of the study population

The flow chart of the study is depicted in Fig. 1. The characteristics of the 1087 included individuals, both at V1 and V4, are summarized in Table 1. Subjects were relatively young ( $40 \pm 8$  years) and showed no comorbidities at baseline. Overall, eGFR remained stable within the 20-year follow-up in the whole population ( $-1.9 \pm 12.2 \text{ ml/min/1.7 m}^2$ ). Between V1 and V4, 146/1087

(13.4%) subjects experienced  $\Delta\text{eGFR} \geq 15 \text{ ml/min/1.7 m}^2$ , 75/1087(6.9%) experienced  $\Delta\text{eGFR} \geq 20 \text{ ml/min/1.7 m}^2$ , and 15/1087 (1.4%) experienced  $\Delta\text{eGFR} \geq 30 \text{ ml/min/1.7 m}^2$  (Supplemental Fig. S1). V4 eGFR was  $<60 \text{ ml/min/1.7 m}^2$  in 15/1087 individuals.

Individuals who experienced  $\Delta\text{eGFR} \geq 15 \text{ ml/min/1.7 m}^2$  had a significantly lower eGFR at V4 ( $77.7 \pm 14.8$  vs  $91.7 \pm 11.2 \text{ ml/min/1.7 m}^2$ ,  $P < .001$ ) despite a higher baseline eGFR ( $99.5 \pm 13.3$  vs  $90.5 \pm 13.2 \text{ ml/min/1.7 m}^2$ ,  $P < .001$ ). There was no demographic difference at baseline between the individuals who further experienced  $\Delta\text{eGFR} \geq 15 \text{ ml/min/1.7 m}^2$  and those who did not (Table 1). At V4, individuals who experienced  $\Delta\text{eGFR} \geq 15 \text{ ml/min/1.7 m}^2$  showed higher rates of antihypertensive drugs use (diuretics and beta-blockers) as well as an increasing trend in urine albumin to creatinine ratio (Table 1). There was no difference in terms of cardiovascular characteristics at V4 between the two groups (Supplemental Table S1).

### Heritability

First, we aimed to assess the heritability of new-onset kidney function decline using the genetic intrafamilial correlations calculated from the GWAS data of our study cohort. Our model allowed us to decompose the total variance of eGFR decline in three components: heritability, common environment effect, and residual effect (i.e. personal environment). We found moderate heritability (30.23%) for eGFR slope between V1 and V4. Common environment and residual effects accounted for 3.76% and 66.01%, respectively.

Table 1: Characteristics: of the study population and according to eGFR decline status.

	Overall (n = 1087)	$\Delta$ eGFR < 15 ml/min/1.7 m <sup>2</sup> (n = 941)	$\Delta$ eGFR $\geq$ 15 ml/min/1.7 m <sup>2</sup> (n = 146)	P value
<b>V1</b>				
Sex female	557(51.2)	490(52.1)	67(45.9)	.19
Age (y)	40 $\pm$ 8	39 $\pm$ 8	40 $\pm$ 8	.64
BMI (kg/m <sup>2</sup> )	24.1 $\pm$ 3.7	24.2 $\pm$ 3.7	23.9 $\pm$ 3.6	.43
Current smoker	236 (21.8)	203 (21.6)	33 (22.76)	.85
NSAID	14 (1.3)	11 (1.2)	3 (2.1)	.90
eGFR (ml/min/1.73 m <sup>2</sup> )	91.7 $\pm$ 13.6	90.5 $\pm$ 13.2	99.5 $\pm$ 13.3	<.001
<b>V4</b>				
Sex female	557 (51.2)	490(52.1)	67 (45.9)	.19
Age (y)	58 $\pm$ 8	58 $\pm$ 8	58 $\pm$ 8	.90
BMI (kg/m <sup>2</sup> )	26.7 $\pm$ 4.9	26.8 $\pm$ 4.9	26.4 $\pm$ 4.4	.37
Current smoker	155 (14.3)	136 (14.5)	19 (13.1)	.75
Diabetes	88 (8.1)	78 (8.3)	10 (6.9)	.67
Anti-diabetic medication	56 (5.4)	47 (5.0)	9 (6.2)	.69
Total cholesterol (g/l)	2.2 $\pm$ 0.4	2.2 $\pm$ 0.4	2.2 $\pm$ 0.4	.14
LDL (g/l)	1.4 $\pm$ 0.3	1.4 $\pm$ 0.3	1.4 $\pm$ 0.3	.19
HDL (g/l)	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	.81
Urine ACR (mg/mmol)	1.7 $\pm$ 10.2	1.3 $\pm$ 4.6	4.3 $\pm$ 25.4	.16
Hypertension	322 (29.6)	273 (29.0)	49 (33.6)	.31
Diuretic	115 (10.1)	91 (9.7)	24 (16.4)	.02
RAAS blocker	75 (6.9)	63 (6.7)	12 (8.2)	.62
Calcium channel blocker	92 (8.5)	79 (8.4)	13 (8.9)	.96
Beta blocker	130 (11.2)	105 (11.2)	25 (17.1)	.05
Statin	204 (18.8)	169 (18.0)	35 (24.0)	.11
NSAID	36 (3.4)	32 (3.4)	4 (2.7)	.87
eGFR (ml/min/1.73 m <sup>2</sup> )	89.8 $\pm$ 12.6	91.7 $\pm$ 11.2	77.7 $\pm$ 14.8	<.001
$\Delta$ eGFR (ml/min/1.73 m <sup>2</sup> )	-1.9 $\pm$ 12.2	1.2 $\pm$ 9.7	-21.8 $\pm$ 6.3	<.001

Variables are expressed as number(percentage) or mean  $\pm$  standard deviation. Multivariable logistic regression model includes the following variables: following variables at V1: age, sex, BMI, current smoker, and eGFR. LDL, low density lipoprotein; HDL, high density lipoprotein; ACR, albumin to creatinine ratio; RAAS, renin angiotensin aldosterone system. NSAID, non-steroidal anti-inflammatory drug.

### Proteins/genes associated with eGFR slope

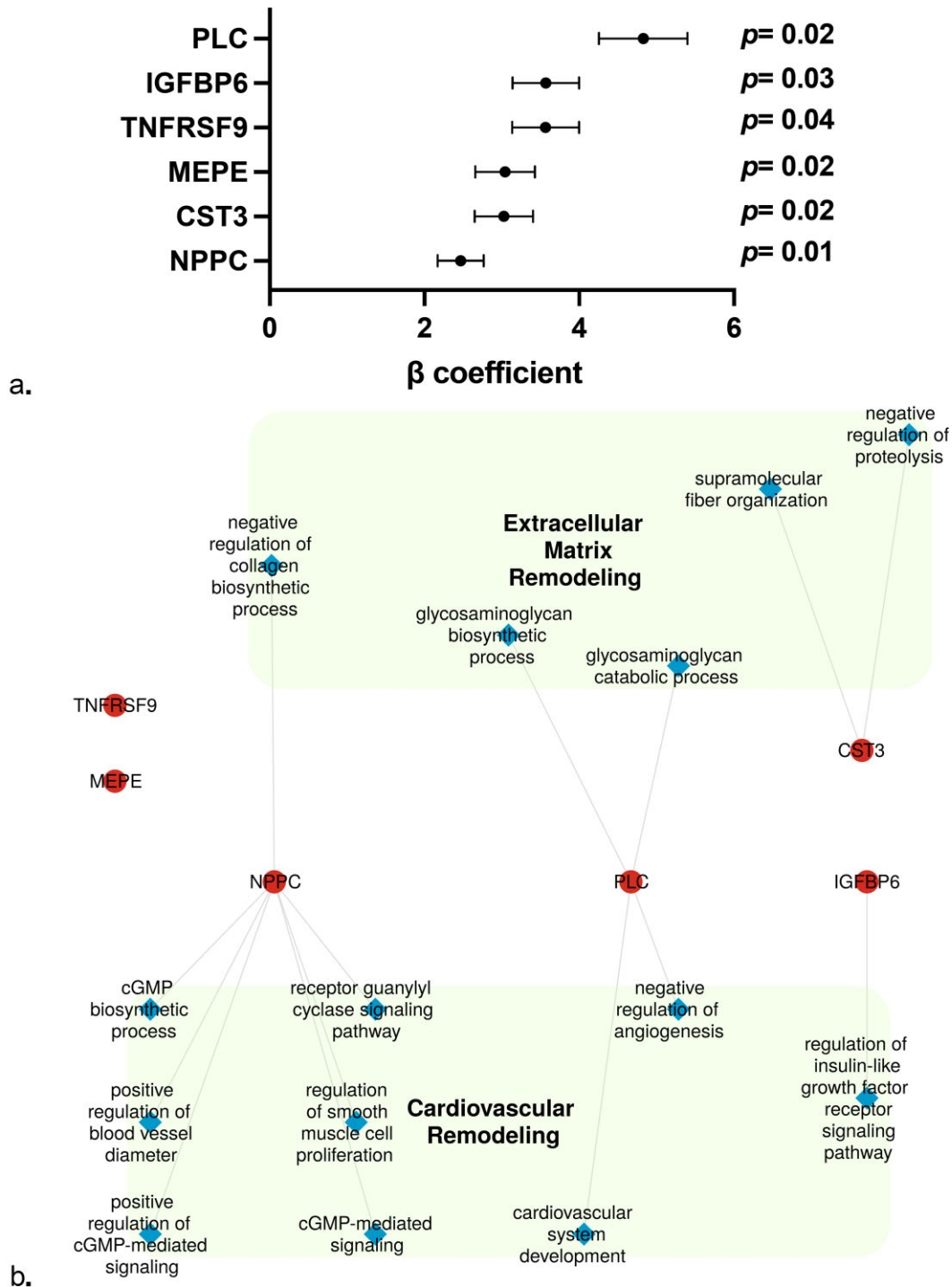
We analysed the proteomic signature associated with eGFR slope from V1 to V4 as a quantitative variable for each individual. The complete list of proteins measured both at V1 and V4 and their respective associations with eGFR slope are shown in the [Supplemental Tables S2](#) and [S3](#). After multivariable selection adjusted for the clinical features, the expression of six out of 445 proteins measured at V1 was found to be significantly and positively associated with eGFR slope and were therefore predictive of further kidney function decline: perlecan (PLC), matrix extracellular phosphoglycoprotein (MEPE), natriuretic peptide precursor C, tumour necrosis factor receptor superfamily member TNFRSF9, insulin-like growth factor binding protein-6, and cystatin C (Fig. 2). Moreover, after multivariable selection adjusted for the clinical features, the expression of 50 out of 445 proteins measured at V4 was found to be significantly and positively associated with eGFR slope, the top ones being PLC and placental growth factor (PGF) (Fig. 3). Conversely, none of the 119765 genes expressions in the 887 individuals with available transcriptomic data at V4 was associated with eGFR slope ([Supplemental Table S4](#)).

The top overrepresented biological processes along with the network analysis results for the association between proteins measured at V1 and V4 and eGFR slope are summarized in [Figs 4](#) and [5](#), and highlight three major features associated with new-onset kidney function decline: cardiovascular remodelling and extracellular matrix remodelling from V1, and inflammation at V4.

### Proteins/genes associated with $\Delta$ eGFR $\geq$ 15 ml/min/1.7 m<sup>2</sup>

To strengthen our analysis, we then compared the proteomic signature between groups of individuals with  $\Delta$ eGFR  $\geq$  15 ml/min/1.7 m<sup>2</sup> (n = 146) and  $\Delta$ eGFR < 15 ml/min/1.7 m<sup>2</sup> (n = 941) between V1 and V4. The complete list of proteins measured both at V1 and V4 and their respective associations with  $\Delta$ eGFR  $\geq$  15 ml/min/1.7 m<sup>2</sup> are shown in the [Supplemental Tables S5](#) and [S6](#). After multivariable selection adjusted for the clinical features, none of the proteins measured at V1 was independently associated with  $\Delta$ eGFR  $\geq$  15 ml/min/1.7 m<sup>2</sup>. Conversely, the expression of 21 out of 445 proteins measured at V4 was found to be significantly and positively associated with new-onset kidney function decline (Fig. 4), the top ones including PLC, five members of the tumour necrosis factor receptor superfamily (TNFR1, TNFR2, TNFRSF9, TNFRSF11A, and LTBR), kallikrein 6 (KLK6), PGF, and MEPE. Areas under the ROC curves to identify  $\Delta$ eGFR  $\geq$  15 ml/min/1.7 m<sup>2</sup> at V4 were  $\geq$ 75% for PLC, three members of the tumour necrosis factor receptor superfamily (TNFR1, TNFR2, and LTBR), ephrin type-B receptor 4 (EPHB4), KLK6, MEPE, and natriuretic peptide precursor C ([Supplemental Fig. S2](#)).

We then analysed the differential genes expression between groups of individuals with  $\Delta$ eGFR  $\geq$  15 ml/min/1.7 m<sup>2</sup> (n = 89) and  $\Delta$ eGFR < 15 ml/min/1.7 m<sup>2</sup> (n = 718) in the 887 individuals with available transcriptomic data at V4. The volcano plot is depicted in [Fig. 5a](#) and shows a slight, yet significant, association between new-onset kidney function decline



**Figure 2:** Associations between proteins levels measured at V1 and eGFR slope. (a) Forrest plot of proteins levels measured at V1 independently associated with eGFR slope. Multivariable linear regression model includes the following variables at V1: age, sex, BMI, current smoker, and eGFR. Dots represent  $\beta$  coefficient and standard error for each protein. P values (with Bonferroni correction) are indicated. (b) Network depiction of proteins co-expression modules that are positively correlated with eGFR slope at V1. Red dots represent proteins and blue diamonds represent biological process connecting the overrepresented proteins. NPPC, natriuretic peptide precursor C; IGFBP6, insulin-like growth factor binding protein-6; CST3, cystatin C.

and the genes expression profile. The expression of 16 out of the 119765 genes was found to be independently associated with new-onset kidney function decline, including six protein-coding genes (Supplemental Table S7). Among the last, two were upregulated (CCL18 and CYP46A1), and four were

downregulated (DNAJC6, SESN3, ETVF1, and NEIL3) in subjects with  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup> (Fig. 5b). Areas under the ROC curves to identify  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup> at V4 were  $\geq 75\%$  for all the genes herein identified (Supplemental Fig. S3).

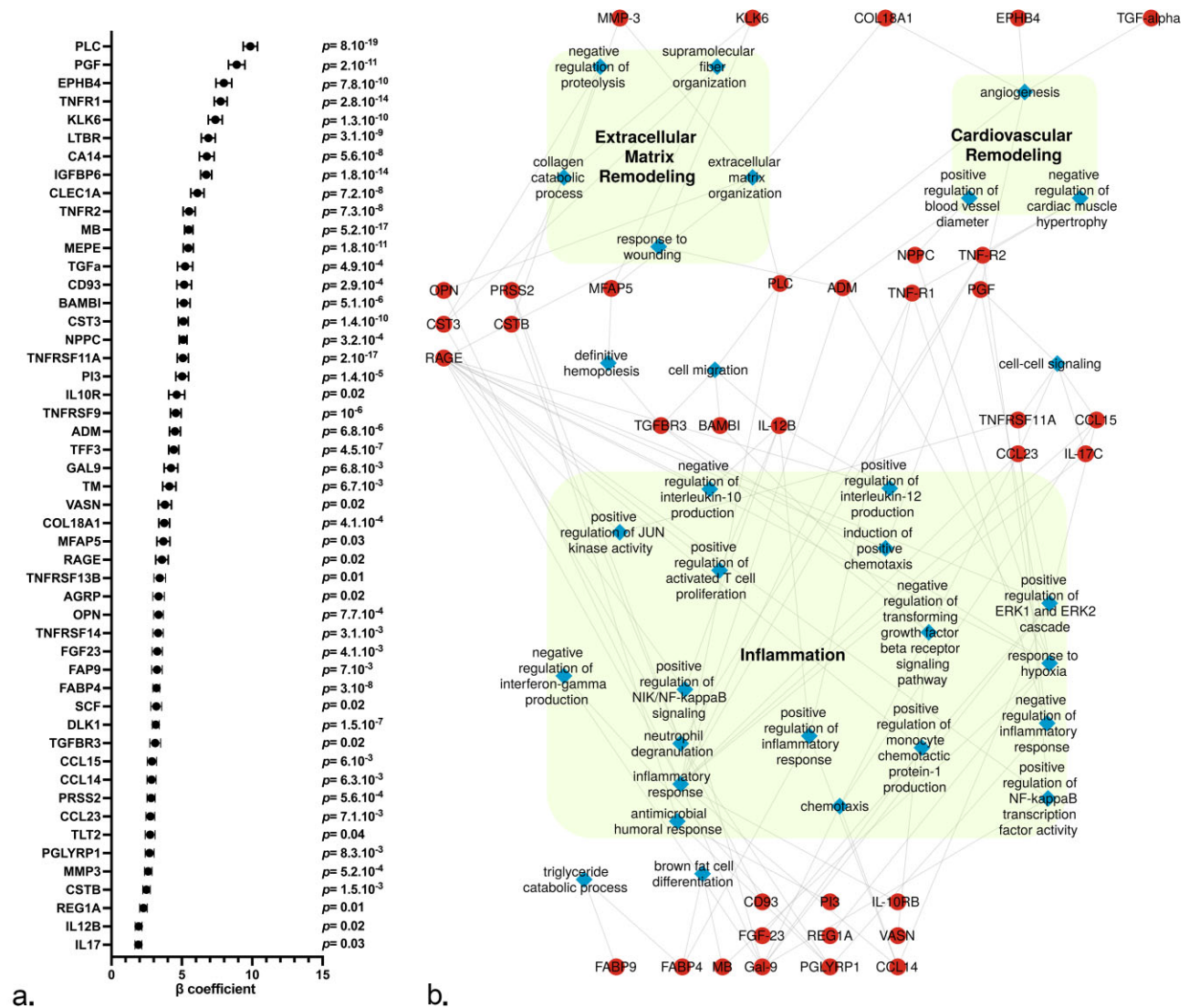


Figure 3: Associations between protein levels measured at V4 and eGFR slope. (a) Forrest plot of protein levels measured at V4 independently associated with eGFR slope. Multivariable linear regression model includes the following variables at V1: age, sex, BMI, current smoker, and eGFR. Dots represent  $\beta$  coefficient and standard error for each protein. P values (with Bonferroni correction) are indicated. (b) Network depiction of protein co-expression modules that are positively correlated with eGFR slope at V4. Red dots represent proteins and blue diamonds represent biological process connecting the overrepresented proteins.

Of particular interest, we noticed that the non-coding MIR1205, which expression is increased at V4 in subjects with new-onset kidney function decline, is predicted to be a potential negative regulator of DNAJC6 expression, which was found to be downregulated in this population. This suggests the existence of a new miRNA-mRNA pair associated with  $\Delta$ eGFR  $\geq 15$  ml/min/1.7 m<sup>2</sup> (Fig. 5c).

## DISCUSSION

The use of a multiomic approach represents a powerful tool to gain an in-depth understanding of the molecular events that modulate several diseases in an unbiased and exhaustive way [28–32]. We took advantage of the STANISLAS cohort to apply this methodology to the setting of new-onset GFR decrease in a unique, longitudinal, large, healthy population without comorbidities at baseline [8].

Our proteomic analysis unravelled 21 and 50 proteins measured at V4 that were independently and positively associated with  $\Delta$ eGFR  $\geq 15$  ml/min/1.7 m<sup>2</sup> and eGFR slope, respectively. Twenty proteins figured in the two lists, the protein having the highest  $\beta$  coefficient being PLC. PLC is a large proteoglycan involved in angiogenesis and extracellular matrix stabilization [33]. Interestingly, PLC plasma level was recently suggested as a surrogate of endothelial damage in CKD patients [34]. We also found PGF, a proatherogenic cytokine and biomarker for cardiovascular events, to be independently associated with eGFR slope, having the second highest  $\beta$  coefficient, while PGF level was previously reported to be increased in patients with decreased kidney function [35]. Taken together, the network analysis supported by our data at V4 highlights both cardiovascular and extracellular matrix remodelling and inflammation as key features of GFR decline.

Interestingly, some of those patterns seem to precede GFR decline. Indeed, six proteins measured at V1 were found to



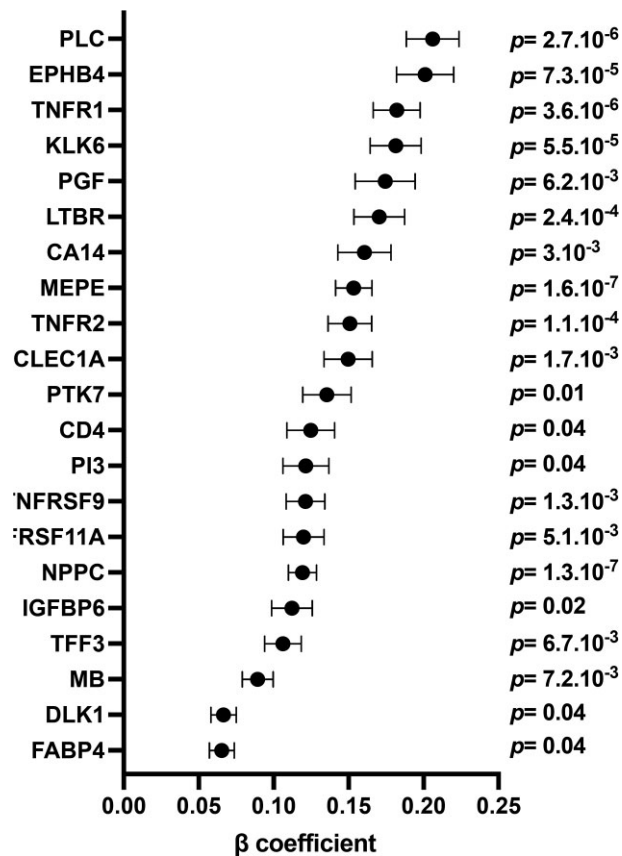


Figure 4: Associations between proteins levels measured at V4 and with  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup>. Forrest plot of proteins levels measured at V4 independently associated with eGFR slope. Multivariable linear regression model includes the following variables at V1: age, sex, BMI, current smoker, and eGFR. Dots represent  $\beta$  coefficient and standard error for each protein. P values (with Bonferroni correction) are indicated.

be associated with eGFR slope, the top one being PLC again. Our network analysis at V1 supports the idea of a cardiovascular and extracellular matrix remodelling that precedes GFR decline and strengthens the idea of a cross-link between kidney disease and accelerated vascular ageing. These findings bring more questions about the *primum movens* between the two phenomena [36].

The data from Dubin et al., who recently described the proteomic patterns associated with GFR decline over 10 years in a cohort of patients with moderate CKD, highlighted the role of ephrin signalling in this setting [10]. Others have underscored the particular role of Ephrin-A4 [37, 38]. Interestingly, we found EPHB4, a receptor for ephrin involved in cardiovascular integrity [39], to be independently associated with GFR decline. In addition, Grams et al. reported associations of proteins levels with incidence of end-stage kidney disease in an older population and identified two members of the tumour necrosis factor receptor superfamily to correlate with renal function impairment [11], while circulating levels of these proteins have been previously reported to increase during later stages of CKD [40]. We report several members of this pro-inflammatory family of proteins to correlate with new-onset kidney function decline.

The familial structure our cohort also allowed to study the heritability for new-onset kidney function decline, which was

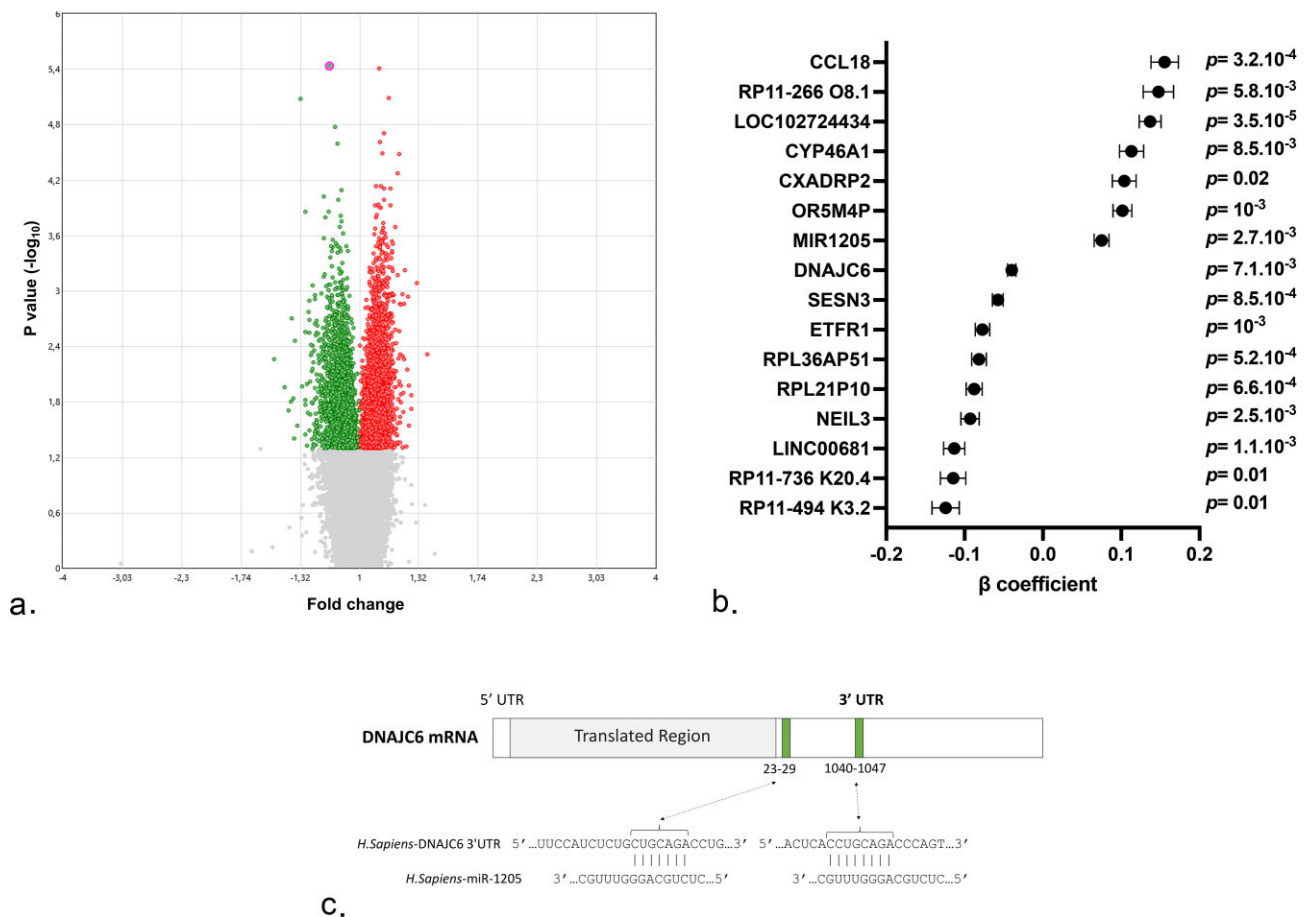
found to be moderate. This suggests that small effects of many gene polymorphisms are involved in this process and that environmental factors are the most potent contributors to this variable.

Several gene expressions were independently associated with  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup> in our study. We report for the first time the positive association between CCL18 gene expression, one of the most highly expressed chemokines in human chronic inflammatory diseases and a biomarker of disease progression, and eGFR decline [41]. Additionally, we report for the first time a negative correlation between SESN3 gene expression, a stress-inducible protein known to protect against oxidative stress and hypoxia, and early kidney function impairment [42]. Similarly, reduced expression of the excision repair enzyme NEIL3, which was previously established as a driver of autoimmunity predisposition, was also associated with loss of renal function [43].

Of interest, both miRDB and TargetsScan (two distinct algorithms used to predict miRNA targets) identified two binding sites for MIR1205 on the 3'-UTR region of the DNAJC6 gene. DNAJC6 belongs to the evolutionarily conserved HSP40 proteins family of proteins and was recently reported to modulate energy metabolism [44]. Taken together with the fact that  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup> is associated with MIR1205 upregulation and DNAJC6 downregulation, these findings suggest the existence of a new miRNA-mRNA pair associated with new-onset GFR decline. Its pathophysiological significance remains to be established by future investigations.

The importance of early identification and intervention for CKD patients has been previously underscored [5]. However, besides limited accuracy, the available biomarkers (such as GFR and albuminuria) represent the consequence of the disease rather than the causal molecular pathophysiology, which further encouraged us to test the ability of the newly uncovered proteins/genes to identify new-onset GFR decline [45]. Interestingly, some of the herein highlighted proteins/genes reached an area over the ROC curve  $>0.75$  to identify  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup> [46]. Whether some of them might represent future biomarkers or potential therapeutic targets to help diagnose kidney disease or slow down its progression remains to be established.

We acknowledge some limitations. First, this was an observational study, hence no causality can be inferred from our results. However, our findings should be regarded as hypothesis generating. Second, we used the eGFR formula to define new-onset kidney function decline and not direct GFR measurements. Nevertheless, we used the CKD-EPI equation, which has been demonstrated to perform more accurately especially at higher ranges of GFR, showing a 70% improvement at  $GFR > 60$  ml/min/1.7 m<sup>2</sup> compared to previous equations [16, 47]. Also, due to the 20-year follow-up design of our study, plasma creatinine levels were measured using different methods at V1 and V4, though the results from Jaffe and enzymatic methods have now been reported not to significantly differ [48, 49]. The relatively low GFR decline over 20 years in our cohort compared to what was previously reported in the general population may be explained by the selected healthy subjects included in our study [50]. Third, all circulating proteins were measured with the Olink® technology, therefore no direct conversion to standard values is possible. Also, transcriptomic data were not available for all participants at V4 which could induce a selection bias, although baseline characteristics were similar in participants and nonparticipants at V4 [13]. Finally, it would be interesting to test if our transcriptomic findings could be



**Figure 5:** Associations between genes expressions at V4 and with  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup>. (a) Volcano plot of genes differentially expressed at V4 when comparing groups of individuals with  $\Delta eGFR < 15$  ml/min/1.7 m<sup>2</sup> (n = 718) and  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup> (n = 89). The x axis represents the mean fold change observed for each gene whereas the y axis displays the log<sub>10</sub> of the P value. Each dot represents one gene. The green dots represent downregulated genes and red dots represent upregulated genes. (b) Forrest plots of genes measured at V4 independently associated with  $\Delta eGFR \geq 15$  ml/min/1.7 m<sup>2</sup>. Multivariable logistic regression model includes the following variables at V1: age, sex, BMI, current smoker, and eGFR. Dots represent  $\beta$  coefficient and standard error for each gene. (c) Predicted duplex formation between miR-1205 and the 3'-UTR of the DNAJC6 mRNA by the miRDB and TargetsCan algorithms.

confirmed at the protein scale, which was not allowed by our approach based on Olink® panels measurements.

Our study provides for the first time a detailed and unbiased proteomic and transcriptomic characterization of new-onset eGFR decline over 20 years of follow-up in an initially healthy population. Our results, which highlight extracellular matrix and cardiovascular remodelling as well as inflammation as major features of early loss of kidney function, lay the foundation to further assess whether the proteins and genes herein identified may represent future biomarkers or potential therapeutic targets of interest to prevent for early CKD.

## SUPPLEMENTARY DATA

Supplementary data are available at [Clinical Kidney Journal](#) online.

## ACKNOWLEDGEMENTS

The authors thank the Centre de Ressources Biologique Lorrain of Centre Hospitalier Régional Universitaire de Nancy for biobank handling. Clinical Trial Registration: NCT01391442.

## AUTHORS' CONTRIBUTIONS

Conceptualization: V.D., P.R.; Formal analysis and investigation: V.D., C.X., I.B.E., E.B., N.G., P.R.; Writing—original draft preparation: V.D.; All authors reviewed and approved the final version of the manuscript.

## FUNDING

The STANISLAS (Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux) study is sponsored by the Nancy Centre Hospitalier Régional Universitaire. This work is supported by the French Ministry of Health 'Programme Hospitalier de Recherche Clinique Inter regional 2013', by the Contrat de Plan Etat-Lorraine and Fonds Européen de Développement Régional Lorraine, and a public grant overseen by the French National Research Agency (ANR) as part of the second 'Investissements d'Avenir' program FIGHT-heart failure (reference ANR-15-RHU-0004) and by the French Plan d'Investissement d'Avenir project 'Lorraine Université d'Excellence', reference ANR-15-IDEX-04-LUE. It is also supported by the European Fibro-Targets

Project (grant agreement no. SP7#602904), European HOMAGE project (grant agreement No. Heart 'Omics' in Ageing, 7th Framework Program grant no. 305507), the MEDIA project Européen 'Cooperation'-Theme 'Health'/FP7-HEALTH-2010-single-stage (reference 261409), FOCUS-MR (reference: ANR-15-CE14-0032-01), ERA-CVD EXPERT (reference ANR-16-ECVD-0002-02), and the Fondation de Recherche en Hypertension Artérielle.

## DATA AVAILABILITY STATEMENT

The data collected for the study, including individual patient data and a data dictionary that define each field in the data set, will be made available as deidentified participant data to researchers who propose to use the data for individual patient data meta-analysis. Data will be shared following approval of the proposal by the corresponding author and a signed data access agreement.

## CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

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Received: 11.4.2024; Editorial decision: 23.6.2024

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