Heinrich Heine
Universität
Düsseldorf

Role of patatin-like phospholipase domain-containing 3 gene for decreasing kidney function in recently diagnosed diabetes mellitus

Oana Patricia Zaharia, Klaus Strassburger, Birgit Knebel, Christian Binsch, Yuliya Kupriyanova, Clara Möser, Kálmán Bódis, Katsiaryna Prystupa, Iryna Yurchenko, Dania Marel Mendez Cardenas, Martin Schön, Christian Herder, Hadi Al-Hasani, Vera Schrauwen-Hinderling, Karin Jandeleit-Dahm, Robert Wagner, Michael Roden

Article - Version of Record

Suggested Citation:
Zaharia, O. P., Strassburger, K., Knebel, B., Binsch, C., Kupriyanova, Y., Möser, C., Bódis, K., Prystupa, K., Yurchenko, I., Mendez Cardenas, D. M., Schön, M., Herder, C., Al-Hasani, H., Schrauwen-Hinderling, V., Jandeleit-Dahm, K., Wagner, R., & Roden, M. (2024). Role of patatin-like phospholipase domain-containing 3 gene for decreasing kidney function in recently diagnosed diabetes mellitus. Diabetes & Metabolic Syndrome, 18(10), Article 103137. https://doi.org/10.1016/j.dsx.2024.103137

Wissen, wo das Wissen ist.

UNIVERSITÄTS- UND LANDESBIBLIOTHEK DÜSSELDORF

This version is available at:

URN: https://nbn-resolving.org/urn:nbn:de:hbz:061-20241202-123505-0

Terms of Use:

This work is licensed under the Creative Commons Attribution 4.0 International License.

For more information see: https://creativecommons.org/licenses/by/4.0

DSX 18 (2024) 103137

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Role of patatin-like phospholipase domain-containing 3 gene for decreasing kidney function in recently diagnosed diabetes mellitus

Oana Patricia Zaharia [a,b,c], Klaus Strassburger [c,d], Birgit Knebel [c,e], Christian Binsch [a,b,c], Yuliya Kupriyanova [b,c], Clara Möser [a,b,c], Kálmán Bódis [a,b,c], Katsiaryna Prystupa [b,c], Iryna Yurchenko [b,c], Dania Marel Mendez Cardenas [b,c], Martin Schön [a,b,c], Christian Herder [a,b,c], Hadi Al-Hasani [c,e], Vera Schrauwen-Hinderling [b,c,f], Karin Jandeleit-Dahm [b,c,g], Robert Wagner [a,b,c], Michael Roden [a,b,c,*], for the GDS Group [1]

[a] Department of Endocrinology and Diabetology, Medical Faculty and University Hospital, Heinrich Heine University, Düsseldorf, Germany
[b] Institute for Clinical Diabetology, German Diabetes Center, Leibniz Institute for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany
[c] German Center for Diabetes Research (DZD e.V.), Partner Düsseldorf, München, Neuherberg, Germany
[d] Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Institute for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany
[e] Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Leibniz Institute for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany
[f] Department of Radiology and Nuclear Medicine, Maastricht University Medical Center, Maastricht, the Netherlands
[g] Department of Diabetes, Central Clinical School, Monash University, Melbourne, Australia

ARTICLE INFO

Keywords:
Nephropathy
Genetic variant
Steatosis

ABSTRACT

Aims: We examined the association of the G allele in the single-nucleotide polymorphism (SNP) rs738409 in the third exon of patatin-like phospholipase domain-containing 3 gene (*PNPLA3)* gene, with chronic kidney disease in diabetes endotypes.
Methods: Participants with recent-onset diabetes (n = 707) from the prospective German Diabetes Study (GDS) underwent cluster assignment, detailed phenotyping, genotyping and magnetic resonance spectroscopy to quantify hepatocellular lipid content (HCL).
Results: Severe insulin-resistant diabetes (SIRD) had the lowest glomerular filtration rates (eGFR) and highest HCL compared to severe insulin-deficient, moderate obesity-related, moderate age-related and severe autoimmune diabetes endotypes (all $p < 0.05$). HCL was negatively associated with eGFR ($r = -0.287$, $p < 0.01$) across all groups. Stratification by G-allele carrier status did not reveal any association between HCL and eGFR among the endotypes. However, the proportion of G-allele carriers increased from 44 % for eGFR >60 ml/min to 52 % for eGFR <60 ml/min ($p < 0.05$).
Conclusions: The *PNPLA3* polymorphism may contribute to declining kidney function independently of liver lipids.

1. Introduction

Over the past decade, several studies demonstrated that nonalcoholic fatty liver disease (NAFLD), which has been recently re-named and re-defined as metabolic dysfunction-associated steatotic liver disease (MASLD) [1], is an important risk factor for other chronic disorders such as cardiovascular disease (CVD), cardiac autonomic neuropathy and chronic kidney disease (CKD) [2,3]. MASLD is not only mutually associated with obesity and type 2 diabetes, but also its progression has been linked to the major diabetes-related complications, CVD and CKD [4,5].

* Corresponding author. Department of Endocrinology and Diabetology Medical Faculty and University Hospital, Heinrich Heine University c/o Auf'm Hennekamp 65, 40225 Düsseldorf, Germany.
E-mail address: michael.roden@ddz.de (M. Roden).

[1] The GDS Group consists of M. Roden (speaker), H. Al-Hasani, B. Belgardt, G. Bönhof, G. Geerling, C. Herder, A. Icks, K. Jandeleit-Dahm, J. Kotzka, O. Kuβ, E. Lammert, W. Rathmann, S. Schlesinger, V. Schrauwen-Hinderling, J. Szendroedi, S. Trenkamp, R. Wagner and their co-workers who contributed to the design and conduct of the GDS.

https://doi.org/10.1016/j.dsx.2024.103137
Received 4 June 2024; Received in revised form 12 September 2024; Accepted 8 October 2024
Available online 14 October 2024
1871-4021/© 2024 Research Trust of DiabetesIndia (DiabetesIndia) and National Diabetes Obesity and Cholesterol Foundation (N-DOC). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

However, the evidence for an independent association between MASLD and CVD remains controversial [6,7], In contrast, emerging data indicate that the association between MASLD and CKD persists regardless of potential confounders such as obesity, hypertension and dysglycemia [8].

The relationship between MASLD and CKD is of high clinical relevance for persons with type 2 diabetes because of their accelerated risk of MASLD progression to hepatic fibrosis and cirrhosis [4,6,9]. Aside from modifiable risk factors leading to positive energy balance, the single-nucleotide polymorphism (SNP) rs738409, which is located in the third exon of the patatin-like phospholipase domain containing 3 (*PNPLA3*) gene, has been implicated in the development and progression of MASLD in humans with diabetes [10]. Moreover, some evidence also suggests an association between *PNPLA3* rs738409 (I148M protein variant; G allele carriers) and risk of CKD, but the mechanisms underpinning this association are poorly understood [11].

Recent studies identified distinct diabetes endotypes (subtypes, clusters) with differences in metabolic features and prevalence of diabetes-related comorbidities and complications [12]. Particularly, the severe insulin resistant diabetes endotype (SIRD) tightly associates with an increased prevalence of both MASLD and CKD [13]. We described that members of SIRD are more frequently carriers of the G allele in rs738409 variant in the *PNPLA3* gene, which further associates with higher circulating non-esterified fatty acids and adipose tissue insulin resistance [14].

In addition to metabolic and vascular factors, adipose-tissue derived cytokines and chemokines likely mediate the crosstalk between MASLD and CKD in diabetes [15], as shown by the correlation between several biomarkers of inflammation and lower baseline kidney function in recent-onset type 2 diabetes [16] and the their upregulation in SIRD [17].

Against this background, we aimed to investigate the role of *PNPLA3* for CKD in the novel diabetes endotypes and MASLD, and the possible associations between the risk allele and biomarkers of CKD at the time of diabetes diagnosis and their changes during the early course of the disease. The novelty of this study lies in the integration of genomic with phenomic data from distinct endotypes at the onset of diabetes. We hypothesized that carriers of the deleterious allele also have lower kidney function than non-carriers.

2. Methods

Participants. This analysis comprises 726 participants with diabetes (type 1 diabetes, n = 234; type 2 diabetes, n = 422) and 70 metabolically healthy humans (control). Persons with diabetes were further stratified according to their diabetes endotype [13] and assigned to either severe autoimmune diabetes (SAID, n = 200), severe insulin-deficient diabetes (SIDD, n = 17), SIRD (n = 53), moderate obesity-related diabetes (MOD, n = 191) or moderate age-related diabetes (MARD, n = 195). A subgroup of participants (n = 424) was prospectively analyzed after 5 years of diabetes duration.

All volunteers were recruited from the Düsseldorf cohort of the prospective multicenter German Diabetes Study (GDS), which includes persons with recently diagnosed diabetes and glucose tolerant humans. The GDS is approved by the ethics committee of Heinrich-Heine University of Düsseldorf (reference number 4508), registered at Clinicaltrials.gov (Identifier number: NCT01055093) and performed according to the Declaration of Helsinki as reported previously [18]. Briefly, diagnosis of diabetes was based on current criteria of the American Diabetes Association [19]. The control group comprises of healthy humans presenting neither with dysglycemia, excluded by a 75 g oral glucose tolerance test [19], nor with first-degree relatives with known diabetes. Exclusion criteria for all participants comprise: pregnancy, acute or severe chronic heart diseases, and immunosuppressive treatment. Known severe kidney diseases as defined by chronic GFR <60 ml*min^{-1}*1.73 m^{-2}, liver diseases as defined by clinical signs or transaminases >2-fold above the upper limit of the normal ranges as well as relevant alcohol use (>30 g/day in men, >20 g/day in women) were also exclusion criteria.

Laboratory analyses. Blood samples drawn at study inclusion were analyzed in a centralized lab as described [18]. Glutamic acid decarboxylase antibodies (GADA) were measured systematically in all participants as described previously [20]. High-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, IL18, soluble E-selectin (sE-selectin) and ICAM-1 intercellular adhesion molecule 1 (ICAM-1) were measured as described [16,21].

Calculations. Steatosis risk was assessed by the Dallas steatosis index (DSI) and the fatty liver index (FLI), whereas liver fibrosis risk was assessed by fibrosis-4 (FIB-4) and Aspartate transaminase to Platelet Ratio Index (APRI) using anthropometric and routine laboratory parameters [22–24]. The estimated glomerular filtration rate (eGFR) was computed based on creatinine and cystatin C and nephropathy was subsequently categorized as normal function (stage 1, eGFR >90 ml*min^{-1}*1.73 m^{-2}), stage 2 CKD (eGFR 60–90 ml*min^{-1}*1.73 m^{-2}) and stage 3 CKD (eGFR <60 ml*min^{-1}*1.73 m^{-2}), while microalbuminuria was defined as urinary albumin levels between 30 and 300 mg/l and macroalbuminuria as urinary excreted albumin levels above 300 mg/l according to international criteria [25].

Magnetic resonance spectroscopy (MRS). HCL was quantified using ^{1}H-MRS on a 3-T MR scanner as described [26]. Both water-suppressed and non-suppressed ^{1}H spectra were taken in the identical voxel within the homogeneous part of liver tissue, avoiding major vessels and gallbladder, with a volume of interest of 25*25*25 mm^{3}. HCL content was calculated from the methylene peak at 1.3 ppm in water-suppressed MRS, relative to the sum of the methylene and water peaks at 4.7 ppm in non-suppressed MRS.

Genotyping. Genomic DNA was extracted from whole blood and genotyping was performed using real-time polymerase chain reaction-based allelic discrimination with probe-based genotyping assay for the rs738409 SNP in the *PNPLA3* gene (Thermofisher, Darmstadt, Germany) as described [14]. Genotypes containing the minor allele C/G and G/G were pooled for the subsequent analyses due to the low number of homozygous carriers.

Statistics. The k-means clustering via nearest centroid approach, assigned each participant to a predefined cluster based on an algorithm including age, BMI, glycemia and homeostasis model estimates (HOMA2-IR, HOMA2-B) and GADA [13].

Data are presented as mean (standard deviation) for continuous variables and percentages (%) for categorical variables. Skewed data were log-transformed before analysis. Associations between parameters have been evaluated using Spearman and adjusted (partialized) Spearman correlation coefficients (r) and corresponding P values. P values < 5 % were considered to indicate statistically significant differences or correlations.

Multiple linear regression models were employed to examine the interaction among estimated glomerular filtration rates (eGFR), fatty liver index (FLI), and biomarkers for inflammation. The analyses assessed the predictive capacity of these biomarkers as independent variables in relation to the onset of diabetes-related complications, which were treated as dependent variables. Statistical analyses were performed with SAS (version 9.4; SAS Institute, Cary, NC) and R software (version R-4.3.2). Figures were drawn using GraphPadPrism (version 8.1; GraphPad Software, San Diego, CA, USA) and R software.

3. Results

3.1. Differences in hepatic lipid content among diabetes endotypes

Characteristics of the participants stratified according to the G-allele of the SNP of the *PNPLA3* gene and to diabetes diagnosis (Table 1) and diabetes endotype (Table S1). FLI was higher in SIRD compared to CON, MARD, SAID (all p < 0.001) and SIDD (p = 0.04) and tended to be higher

Table 1
Characteristics of the participants stratified according to diabetes diagnosis and G allele carrier status of the single-nucleotide polymorphism (SNP) rs738409 in the patatin-like phospholipase domain containing 3 (PNPLA3) gene.

	CON		T1D		T2D	
	G allele carriers	Non-carriers	G allele carriers	Non-carriers	G allele carriers	Non-carriers
N	25	45	106	128	199	223
Age (years)	46.6 ± 13.7	49.3 ± 13.9	37.6 ± 12.4	37.0 ± 11.7	52.9 ± 10.5	53.7 ± 10.0
Sex (% female)	7 (28)	14 (31)	50 (47)	53 (41)	77 (39)	71 (32)
BMI (kg/m^2)	28.5 ± 6.3	28.8 ± 5.1	25.9 ± 5.1	24.8 ± 3.8	32.1 ± 5.7	31.6 ± 6.1
HbA1c (%)	5.3 ± 0.2	5.3 ± 0.3	6.6 ± 1.2	6.6 ± 1.2	6.4 ± 1.0	6.4 ± 0.9
HOMA2-B (a.u.)	117 ± 43	122 ± 34	48 ± 36	47 ± 29	104 ± 47	98 ± 40
HOMA2-IR (a.u.)	1.4 ± 0.7	1.5 ± 0.7	1.1 ± 1.1	0.9 ± 0.6	2.8 ± 1.1	2.7 ± 1.3
eGFR ($ml*min^{-1}*1.73\ m^{-2}$)	95.9 ± 13.7	91.5 ± 14.9	98.2 ± 16.0	100 ± 13.0	87.8 ± 16.5	87.8 ± 14.6
Cystatin C (mg/l)	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	0.9 ± 0.2
Microalbuminuria (n,%)	1 (4)	1 (2)	5 (5)	6 (5)	16 (8)	28 (12)
Macroalbuminuria (n,%)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1)	0 (0)
ALT (U/l)	27.8 ± 15.8	29.5 ± 22.3	24.4 ± 13.9	24.1 ± 20.5	35.9 ± 18.4	33.6 ± 19.5
AST (U/l)	25.0 ± 6.7	26.0 ± 13.6	21.8 ± 8.1	21.3 ± 8.7	25.8 ± 11.2	25.4 ± 11.5
GGT (U/l)	26.0 ± 19.0	27.6 ± 19.7	27.3 ± 31.7	18.7 ± 12.2	38.0 ± 29.9	49.5 ± 67.9
HCL (%)	4.6 ± 7.0	3.4 ± 4.7	1.6 ± 2.9	2.3 ± 6.3	9.8 ± 9.2	7.2 ± 6.8
FLI (a.u.)	47.8 ± 30.0	49.1 ± 34.9	32.4 ± 29.9	24.1 ± 25.7	74.5 ± 24.3	71.0 ± 28.6
DSI (a.u.)	−0.9 ± 1.5	−0.9 ± 1.4	−1.1 ± 1.4	−1.4 ± 1.2	0.9 ± 1.2	0.6 ± 1.3
FIB4 (a.u.)	1.0 ± 0.3	1.1 ± 0.4	0.8 ± 0.4	0.8 ± 0.4	1.1 ± 0.6	1.1 ± 0.5
APRI (a.u.)	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
hsCRP (mg/dl)	0.1 ± 0.2	0.2 ± 0.3	0.4 ± 1.5	0.2 ± 0.3	0.4 ± 0.5	0.4 ± 0.6
IL6 (pg/ml)	NA	NA	1.6 ± 1.4	1.2 ± 0.8	2.4 ± 1.5	2.3 ± 4.4
IL18 (pg/ml)	NA	NA	322 ± 174	263 ± 135	334 ± 173	299 ± 118
E-selectin (ng/ml)	NA	NA	39.4 ± 21.3	38.1 ± 17.0	46.3 ± 20.3	42.5 ± 19.1
ICAM-1 (ng/ml)	NA	NA	242 ± 77	228 ± 64	256 ± 77	250 ± 77
Lifestyle modification only (n)	25	45	4	11	74	92
Insulin (n)	0	0	95	104	16	16
Metformin (n)	0	0	4	11	98	102
Other (n)	0	0	3	2	11	13

Data are shown as absolute numbers, percentages, mean ± standard deviation, as applicable. Abbreviations: ALT, alanine aminotransferase; APRI, AST to Platelet Ratio Index; AST, aspartate aminotransferase; BMI, body mass index; CON, control group; DSI, Dallas steatosis index; eGFR, estimated glomerular filtration rate; FIB4, fibrosis 4 index; FLI, fattly liver index; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin A1c; HOMA2, homeostatic model assessment for insulin resistance (IR) and beta cell function (B); hsCRP, high-sensitivity C-reactive protein; IL, interleukin; T1D, type 1 diabetes; T2D, type 2 diabetes.

compared to MOD ($p = 0.07$). In a representative subcohort with available ^{1}H-MRS ($n = 226$), HCL was higher in SIRD than in CON, MARD, SAID ($p < 0.001$) and MOD ($p = 0.028$), but similar to SIDD ($p = 0.21$). Accordingly, prevalence of steatosis by HCL was 80 % in SIRD, 59 % in MOD, 31 % in MARD, 25 % in SIDD, 23 % in CON and only 5 % in SAID. FLI values > 60 predicted the presence of steatosis (HCL >5.56 %) with a sensitivity of 93 % and a specificity of 70 %.

3.2. Distribution of the rs738409 variant of the PNPLA3 gene across diabetes endotypes

There was no difference in the prevalence of G-allele carriers between participants with type 1 diabetes, type 2 diabetes or normoglycemia (Fig. 1A). Similarly, there were no differences in eGFR between carriers and non-carriers neither at baseline (Fig. 1B) nor in the change of eGFR during 5 years of disease duration (Fig. 1C). These results remained unchanged after adjustments for HOMA-IR.

Endotype stratification revealed a lower prevalence of the common C/C genotype in SIRD than in CON, SAID, MOD and MARD (all $p < 0.05$) except for SIDD ($p = 0.68$; Fig. 1D). When considering the endotypes individually, there were no differences in eGFR between carriers and non-carriers neither at baseline (Fig. 1E) nor in the change of eGFR after 5 years of disease duration (Fig. 1F).

3.3. Association between hepatic lipid content and kidney function in diabetes endotypes

As assessed from eGFR, the prevalence of stage 2 CKD was 67 % in SIRD, 56 % in MARD, 45 % in CON, 44 % in MOD 25 % in SAID and 17 % in SIDD. Stage 3 CKD was present in 15 % SIRD, 3 % MARD, 2 % MOD, 1 % CON, and 0 % in SAID or SIDD. HCL was inversely associated with eGFR across all groups ($r = 0.29$, $p < 0.05$, Fig. 2), which also held true for FLI ($r = 0.47$, $p < 0.05$).

Stratification by G allele carrier status did not reveal any association between FLI and eGFR specific to any of the diabetes endotypes. However, with declining eGFR the proportion of G-allele carriers increased from 44 % for eGFR >60 $ml*min^{-1}*1.73\ m^{-2}$ to 52 % for eGFR <60 $ml*min^{-1}*1.73\ m^{-2}$ ($p < 0.05$, Fig. 3A), while G allele carriers had higher FLI values than non-carriers in the group featuring eGFR >90 $ml*min^{-1}*1.73\ m^{-2}$ ($p < 0.05$; Fig. 3B).

We performed a partial correlation analysis to examine the relationship between eGFR and HCL while controlling for insulin resistance (HOMA2-IR). The unadjusted Pearson correlation between eGFR and HCL ($r = -0.29$, $p < 0.001$) suggests that the observed correlation between eGFR and liver lipid content maybe at least partly mediated by the prevailing insulin resistance.

3.4. Role of pro-inflammatory biomarkers for CKD and hepatic steatosis

Overall, hsCRP was positively associated with HCL ($r = 0.386$, $p <$

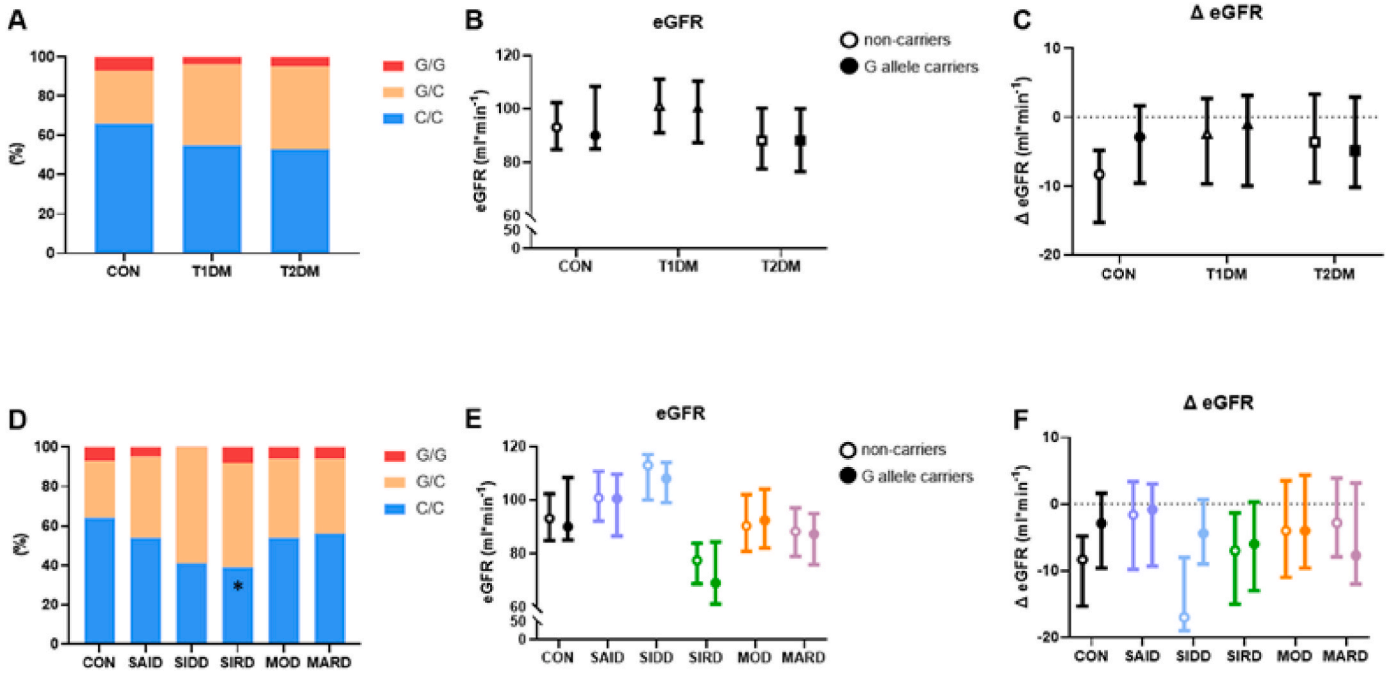

Fig. 1. Data are presented as percentages or mean and standard deviation. Figures depict differences in G allele carrier status (panels A, C), and estimated glomerular filtration rates (eGFR) in metabolically healthy humans (CON, circles), type 1 diabetes (T1DM, triangles) and type 2 diabetes (T2DM, squares) (panel B, C) as well as in diabetes endotypes: severe autoimmune diabetes (SAID) in indigo, severe insulin deficient diabetes (SIDD) in light blue, severe insulin-resistant diabetes (SIRD) in green, moderate obesity-related diabetes (MOD) in orange and moderate age-related diabetes (MARD) in pink (panel D, E). Δ eGFR refers to the difference between eGFR after 5 years and eGFR at study inclusion (baseline).
Full shapes represent carriers of the variant (G-allele) while empty shapes represent non-carriers. *, $p < 0.05$.

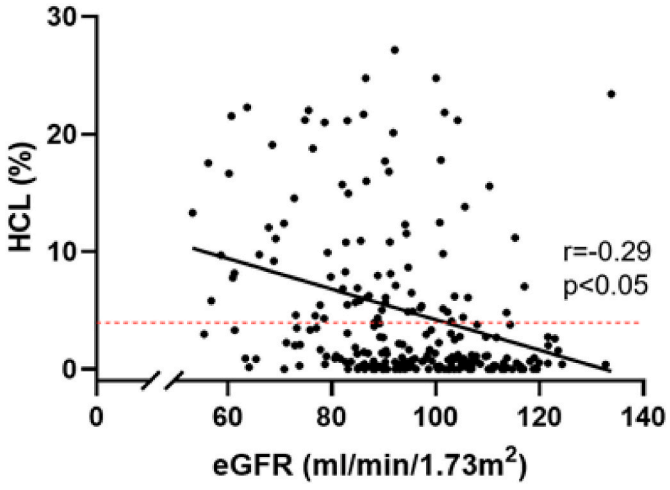

Fig. 2. Scatterplot showing the association of renal function with steatosis in the study population. Dotted line refers to cut-off values for MASLD.

0.001), but not with eGFR ($r = -0.068$, $p = 0.07$). Among various biomarkers of inflammation and endothelial cell adhesion (hsCRP, IL-6, IL-18, E-selectin, ICAM-1), only IL-6 was found to be higher in G-allele carriers that in non-carriers with SIRD (Table S1).

Fig. 4 shows the tridimensional relationship between pro-inflammatory markers, FLI and eGFR across all groups. Trilinear regression models showed a weak, yet statistically significant (all $p < 0.05$) interrelation with IL-6 ($r = -0.001$, Fig. 4A) and ICAM-1 ($r = 0.033$, Fig. 4C) in type 1 diabetes, but not in type 2 diabetes (Fig. 4B–D).

4. Conclusions

This study shows that higher hepatic lipid content is associated with lower kidney function across this study population. However, the *PNPLA3* polymorphism does not directly affect this association in any diabetes endotype, neither at disease onset, not within the first 5 years after diagnosis.

The finding of a strong correlation between markers of liver steatosis and eGFR among diabetes endotypes extends the conclusion of previous meta-analyses that MASLD identifies individuals at increased risk of CKD [8,27,28]. This suggests a specific role of the hepato-renal axis independently of glucometabolic differences operating even before the onset of advanced liver diseases [29].

The observed absence of a direct effect of the *PNPLA3* polymorphism rs738409(G) on kidney function in any diabetes endotype contrasts with the association of the I148M variant of *PNPLA3* with CKD, occurring independently of common risk factors for kidney disease and severity of MASLD in some previous studies [11,30]. PNPLA3 expression levels were particularly high in renal podocytes [11]. Datasets on human kidney biopsy material also indicate relevant PNPLA3 expression in the proximal tubule cells (Humphreyslab.com online resource). Furthermore, a specific subgroup of persons with MASLD, carrying the *PNPLA3* rs738409 G allele, was found to be at higher risk of early glomerular and tubular damage [31]. From another study, the authors concluded that MASLD remains the main determinant of decline in kidney function in overweight children, while the *PNPLA3* rs738409 variant has a small, if any, impact [32]. Surprisingly, their subsequent study found that already prediabetic state negatively affects renal function in children with obesity, with a greater effect in those carrying the G allele [33]. The differences between the different studies likely results from specific features of the cohorts such as age, diabetes and degree of CKD and/or MASLD. Our study included individuals with normal glucose tolerance and those with metabolically well-controlled diabetes, short known diabetes duration and no severe liver and kidney disease. Thus, we may

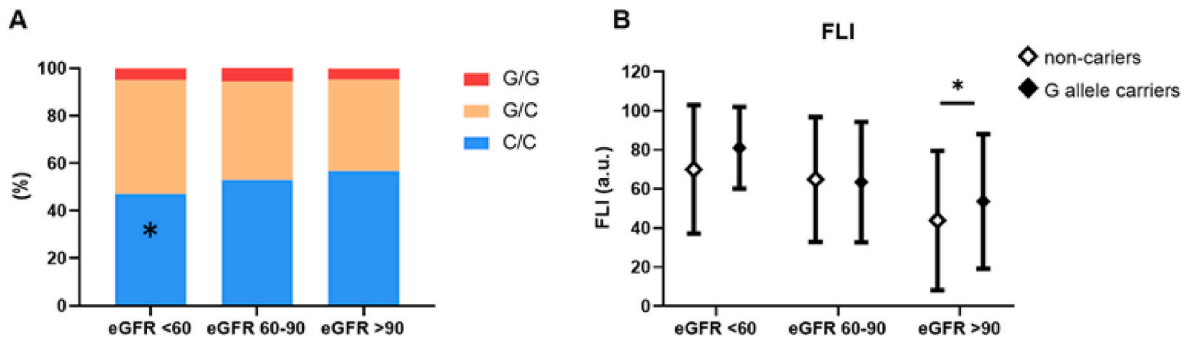

Fig. 3. Data are presented as percentages or mean and standard deviation. Figures depict differences in G allele carrier status (panel A), and fatty liver index (FLI, panel B) in categories based on estimated glomerular filtration rates (eGFR). Full shapes represent carriers of the variant (G-allele) while empty shapes represent non-carriers. *, $p < 0.05$.

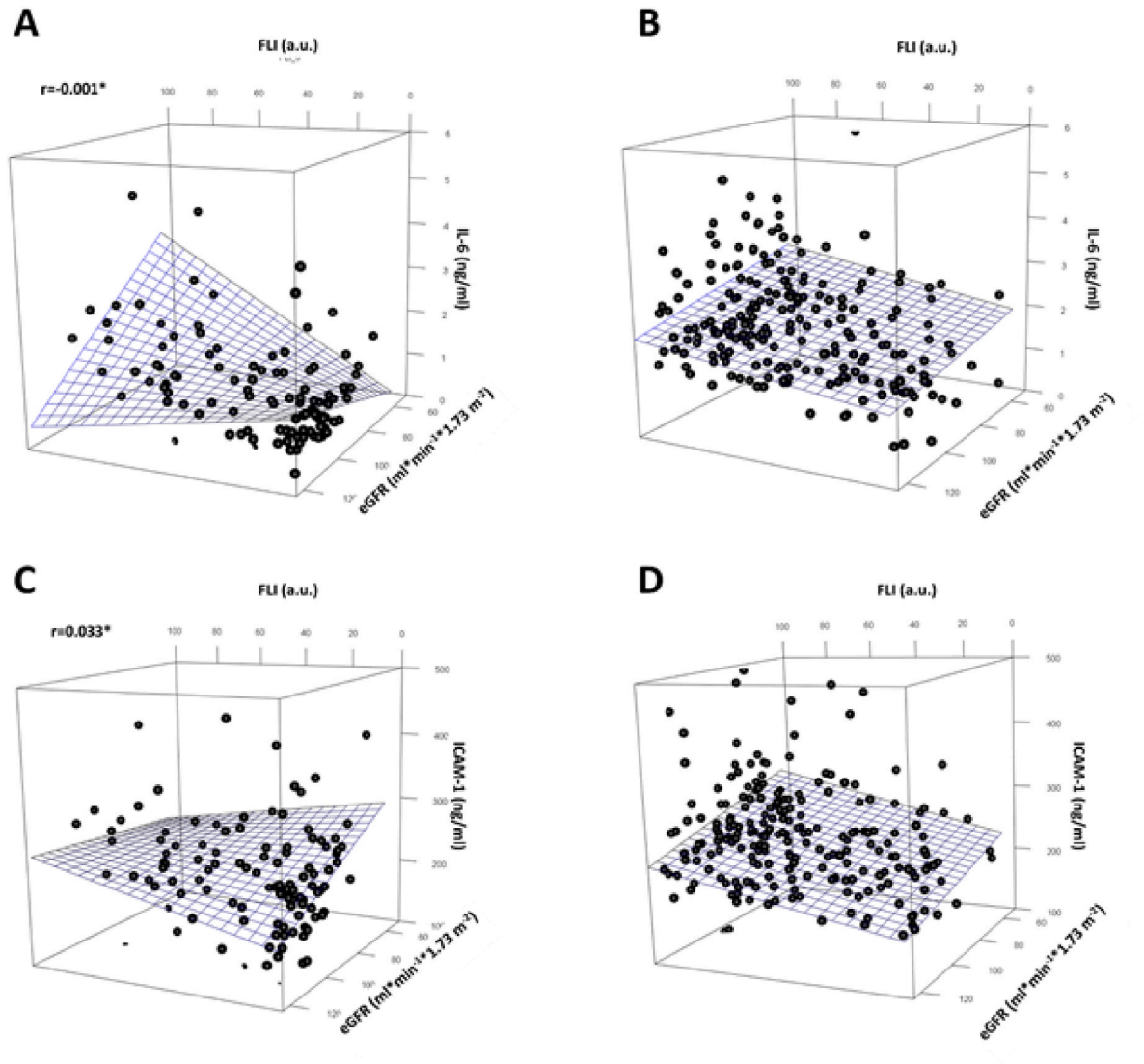

Fig. 4. Trilinear regression models showing the interrelation between estimated glomerular filtration rates (eGFR), fatty liver index (FLI) and interleukin 6 (IL-6, panels A, B), ICAM-1 (panel C, D) in type 1 diabetes (panels A,C) and type 2 diabetes (panels B, D) respectively. *, $p < 0.05$.

conclude that the *PNPLA3* genotype is at least not the main driver of CKD during the early course of diabetes or its endotypes.

Indeed, the mechanisms suggested to underlie an association between the G allele of rs738409 and decreasing kidney function are yet unclear. For example, nephrotoxic effects occurring via activation of renal pericytes, which play a key role in angiogenesis and in regulating renal medullary and cortical blood flow, can promote renal fibrogenesis and glomerulosclerosis [8]. Alternatively, glomerular podocytes may accumulate lipid droplets, which have been implicated in the pathogenesis of CKD [34]. Also, dysregulation of proximal tubule transport

driven by misexpression of *PNPLA3* may lead to abnormalities in electrolyte or acid-base-homoeostasis and glucosuria [35].

Of note, other - indirect - effects related to inflammation and fibrosis are largely unexplored. A recent meta-analysis showed that increased concentrations of pro-inflammatory mediators such as hsCRP and IL-6 were associated with increased risk of MASLD and diabetes and may therefore add to biomarker panels for early MASLD diagnosis and MASLD progression [15,36]. Several of these biomarkers also associate with lower eGFR in recent-onset diabetes [16]. The present study detected a three-way interaction between CKD, steatosis and inflammation indicating that low-grade (subclinical) inflammation orchestrates this interorgan communication at least in persons with diabetes [15,36]. However, the absence of differences between carriers and non-carriers with respect to pro-inflammatory biomarkers in any of the diabetes endotypes suggest that this relationship occurs independently of the *PNPLA3* SNP carrier status. Only in the presence of severe insulin resistance, the carriers of the G-allele exhibited increased circulating IL-6 levels, which are known to associate with higher adipose tissue lipolysis, which promotes hepatic lipogenesis, gluconeogenesis and insulin resistance [37]. This finding supports previous observations suggesting *PNPLA3*-induced susceptibility to hepatic steatosis is at least partly mediated by adipose tissue dysfunction, which might contribute to MASLD via excessive lipolysis and augmented flux of nonesterifed fatty acids to the liver [14]. Our analyses further suggest a relevant role for insulin resistance driving the relationship between CKD and MASLD.

One recent review proposed that the association between *PNPLA3* and progression of MASLD to fibrosis seems to predispose to the development of CKD [2]. It has been also postulated that the prevalence of impaired renal function in persons with MASLD is dependent on the severity of liver disease, particularly in the presence of diabetes [38]. Indeed, the *PNPLA3* variant may have detrimental impact on eGFR even in middle-aged individuals with metabolic dysfunction independently of established risk factors and ethnicity [34]. Of note, most studies in adults showing the association between carriers of the G allele and CKD were described in Asian populations [30,31]. The present study cannot confirm these findings in people with short known diabetes duration and early MASLD, but cannot exclude a relevant role of the G allele during long-standing diabetes and with progression of both MASLD and CKD [33].

Of note, disease duration and progression are particularly important for future therapeutic strategies that target both liver lipid reduction as well as preservation of renal function in order to prevent clinical outcomes. Among individuals with type 2 diabetes, specifically sodium glucose transport 2 inhibitors (SGLT2i) consistently reduce the risk for renal outcomes [39], but also lead to improved adipose tissue function and decreased HCL [40]. In the present study, only less than 5 % of the persons with diabetes were on SGLT2i, which did not allow further subgroup analysis, but unlikely affects the overall results also because of the relatively short observation period.

The present analysis benefits from the comparatively large size of the comprehensively phenotyped GDS cohort with a defined short known diabetes duration, which reduces confounding by chronic diabetes-related alterations such as - but not only limited to – hyperglycemia, dyslipidemia and low-grade inflammation. The GDS cohort further allows to examine the novel diabetes endotypes and monitor changes during first 5 years of disease. This study uniquely demonstrates that increased HCL is associated with reduced eGFR across all diabetes endotypes, extending beyond the SIRD endotype. Additionally, it highlights the role of the G-allele in the *PNPLA3* gene as a potential contributor to declining kidney function, independent of its direct effect on the relationship between HCL and eGFR. Nevertheless, the exclusion criteria of GDS limits the generalizability of the present data for humans without severe liver and kidney diseases (GFR<60 ml/min). This study also cannot address effects in people with severe MASLD and/or CKD. Finally, all study participants are of European descent, so that this study cannot examine the impact of ethnic disparities, e.g. regarding MASLD prevalence and severity, which has been reported to be highest in Hispanics and lowest in African descent [41]. Overall, the lack of effect seen in the individual phenotypes can be attributed to the low sample after subclassification.

In conclusion, presence of hepatic steatosis is associated with lower kidney function across different diabetes endotypes already at the onset of diabetes. While this association occurs independently of the *PNPLA3* polymorphism rs738409(G), there might be a detrimental role for the PNPLA3 gene variation in progressing CKD, occurring independently of diabetes endotype.

Advances in precision medicine could make targeting specific SNPs like rs738409 a pivotal strategy for identifying humans at higher risk for CKD, enabling early intervention and tailored therapies, ultimately improving outcomes.

Authors' contribution

OPZ drafted the manuscript. KS and CB performed the statistical analyses. BK, HAH performed and interpreted the genetic analyses. OPZ, MS, CM, IY, DMMC, KB, and KP performed metabolic analyses and clinical examinations. YK and VSH performed and analyzed the metabolic imaging data. CH analyzed the biomarkers of inflammation. KJD, RW and MR contributed to the discussion and reviewed/edited the manuscript. All authors critically reviewed the manuscript. MR is the guarantor of this work 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. All authors read and approved the final manuscript.

Prior presentations

Parts of this work were presented at the Scientific Session of the American Diabetes Association, San Diego, CA, USA (2023) and at the Annual Meeting of the European Association of the Study of Diabetes, Hamburg, Germany (2023).

Conflict of interest

MR reports fees for lecturing and/or advisory board activities from Astra-Zeneca, Boehringer-Ingelheim Pharma, Echosens, Eli Lilly, Madrigal, MSD and Novo Nordisk as well as for investigator-initiated studies from Boehringer-Ingelheim, Novo Nordisk and Nutricia/Danonone. OPZ reports lecture fees from Sanofi. RW reports lecture fees from Novo Nordisk, Sanofi and Eli Lilly and served on an advisory board for Akcea Therapeutics, Daiichi Sankyo, Sanofi, Eli Lilly and Novo Nordisk. KS, BK, YK, CB, DM, KP, IY, KB, CH, VSH, HAH, KJD, CM, MS declare no competing interests.

Data availability

The datasets used for the current study are available from the German Diabetes Study (GDS; PI: M.R.) upon reasonable request.

Funding

The German Diabetes Study was initiated and financed by the German Diabetes Center (which is funded by the German Federal Ministry of Health and the Ministry of Culture and Science of the state of North Rhine-Westphalia), the German Diabetes Association, the German Federal Ministry of Education and Research (to the German Center for Diabetes Research, DZD), the German Research Foundation (DFG, CRC-SFB 1116) and the Schmutzler Stiftung. The research of MR is further supported by grants from the European Community (HORIZON-HLTH-2022-STAYHLTH-02-01: Panel A) to the INTERCEPT-T2D consortium and the German Science Foundation (DFG; RTG/GRK 2576 vivid, Project 3 and 493659010 Future4CSPMM). The research of OPZ is supported

by grants from the European Foundation for the Study of Diabetes (EFSD Rising Star Award) and the German Diabetes Association (DDG Adam Heller Prize). The project has received funding from the programme "Profilbildung 2020″, an initiative of the Ministry of Culture and Science of the State of Northrhine Westphalia (PROFILNRW-2020-107-B). The sole responsibility for the content of this publication lies with the authors. The funding sources had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank the staff of the German Diabetes Center (DDZ) for their excellent support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2024.103137.

References

[1] Rinella ME, Lazarus JV, Ratziu V, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023.
[2] Ciardullo S, Ballabeni C, Trevisan R, Perseghin G. Liver stiffness, albuminuria and chronic kidney disease in patients with NAFLD: a systematic review and meta-analysis. Biomolecules 2022;12:105.
[3] Ziegler D, Strom A, Kupriyanova Y, et al. Association of lower cardiovagal tone and baroreflex sensitivity with higher liver fat content early in type 2 diabetes. J Clin Endocrinol Metab 2018;103:1130–8.
[4] Roden M, Shulman GI. The integrative biology of type 2 diabetes. Nature 2019; 576:51–60.
[5] Pafili K, Roden M. Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans. Mol Metabol 2020:101122.
[6] Targher G, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus — mechanisms and treatments. Nat Rev Gastroenterol Hepatol 2021;18:599–612.
[7] Henson JB, Roden M, Targher G, Corey KE. Is nonalcoholic fatty liver disease not a risk factor for cardiovascular disease: not yet time for a change of heart. Hepatology 2020;71:1867–9.
[8] Byrne CD, Targher G. NAFLD as a driver of chronic kidney disease. J Hepatol 2020; 72:785–801.
[9] Gancheva S, Jelenik T, Alvarez-Hernandez E, Roden M. Interorgan metabolic crosstalk in human insulin resistance. Physiol Rev 2018;98:1371–415.
[10] Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–5.
[11] Mantovani A, Taliento A, Zusi C, et al. PNPLA3 I148M gene variant and chronic kidney disease in type 2 diabetic patients with NAFLD: clinical and experimental findings. Liver Int 2020;40:1130–41.
[12] Herder C, Roden M. A novel diabetes typology: towards precision diabetology from pathogenesis to treatment. Diabetologia 2022;65:1770–81.
[13] Zaharia OP, Strassburger K, Strom A, et al. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. Lancet Diabetes Endocrinol 2019;7:684–94.
[14] Zaharia OP, Strassburger K, Knebel B, et al. Role of patatin-like phospholipase domain-containing 3 gene for hepatic lipid content and insulin resistance in diabetes. Diabetes Care 2020;43:2161–8.
[15] Xourafa G, Korbmacher M, Roden M. Inter-organ crosstalk during development and progression of type 2 diabetes mellitus. Nat Rev Endocrinol 2024;20:27–49.
[16] Maalmi H, Herder C, Strassburger K, et al. Biomarkers of inflammation and glomerular filtration rate in individuals with recent-onset type 1 and type 2 diabetes. J Clin Endocrinol Metab 2020.
[17] Herder C, Maalmi H, Strassburger K, et al. Differences in biomarkers of inflammation between novel subgroups of recent-onset diabetes. Diabetes 2021;70: 1198–208.
[18] Szendroedi J, Saxena A, Weber KS, et al. Cohort profile: the German diabetes study (GDS). Cardiovasc Diabetol 2016;15:59.
[19] Classification and diagnosis of diabetes: standards of medical care in diabetes-2022. Diabetes Care 2022;45:S17–s38. 2.
[20] Lohmann T, Kellner K, Verlohren HJ, et al. Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). Diabetologia 2001;44: 1005–10.
[21] Schamarek I, Herder C, Nowotny B, et al. Adiponectin, markers of subclinical inflammation and nerve conduction in individuals with recently diagnosed type 1 and type 2 diabetes. Eur J Endocrinol 2016;174:433–43.
[22] Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. Hepatology 2017;66:1486–501.
[23] Kahl S, Strassburger K, Nowotny B, et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS One 2014;9:e94059.
[24] McHenry S, Park Y, Browning JD, Sayuk G, Davidson NO. Dallas steatosis index identifies patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol : the official clinical practice journal of the American Gastroenterological Association 2020;18:2073–2080.e2077.
[25] Levey AS, Coresh J, Balk E, et al. National kidney foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 2003;139:137–47.
[26] Kupriyanova Y, Zaharia OP, Bobrov P, et al. Early changes in hepatic energy metabolism and lipid content in recent-onset type 1 and 2 diabetes mellitus. J Hepatol 2021;74:1028–37.
[27] Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care 2018;41:372–82.
[28] Musso G, Gambino R, Tabibian JH, et al. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. PLoS Med 2014;11:e1001680.
[29] Golestaneh L, Neugarten J. Dual organ duel: the hepatorenal Axis. Adv Chron Kidney Dis 2017;24:253–60.
[30] Oniki K, Saruwatari J, Izuka T, et al. Influence of the PNPLA3 rs738409 polymorphism on non-alcoholic fatty liver disease and renal function among normal weight subjects. PLoS One 2015;10:e0132640.
[31] Sun DQ, Zheng KI, Xu G, et al. PNPLA3 rs738409 is associated with renal glomerular and tubular injury in NAFLD patients with persistently normal ALT levels. Liver Int : official journal of the International Association for the Study of the Liver 2020;40:107–19.
[32] Di Costanzo A, Pacifico L, D'Erasmo L, et al. Nonalcoholic fatty liver disease (NAFLD), but not its susceptibility gene variants, influences the decrease of kidney function in overweight/obese children. In: International journal of molecular sciences; 2019.
[33] Di Sessa A, Russo MC, Arienzo MR, et al. PNPLA3 I148M polymorphism influences renal function in children with obesity and prediabetes. J Ren Nutr : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 2022; 32:670–6.
[34] Mantovani A, Pelusi S, Margarita S, et al. Adverse effect of PNPLA3 p.I148M genetic variant on kidney function in middle-aged individuals with metabolic dysfunction. Aliment Pharmacol Therapeut 2023;57:1093–102.
[35] Chevalier RL. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction. Am J Physiol Ren Physiol 2016;311:F145–61.
[36] Duan Y, Pan X, Luo J, et al. Association of inflammatory cytokines with non-alcoholic fatty liver disease. Front Immunol 2022;13:880298.
[37] Han MS, White A, Perry RJ, et al. Regulation of adipose tissue inflammation by interleukin 6, vol. 117. Proceedings of the National Academy of Sciences; 2020. p. 2751–60.
[38] Nampoothiri RV, Duseja A, Rathi M, et al. Renal dysfunction in patients with nonalcoholic fatty liver disease is related to the presence of diabetes mellitus and severity of liver disease. Journal of clinical and experimental hepatology 2019;9: 22–8.
[39] Cherney DZI, Charbonnel B, Cosentino F, et al. Effects of ertugliflozin on kidney composite outcomes, renal function and albuminuria in patients with type 2 diabetes mellitus: an analysis from the randomised VERTIS CV trial. Diabetologia 2021;64:1256–67.
[40] Kahl S, Ofstad AP, Zinman B, et al. Effects of empagliflozin on markers of liver steatosis and fibrosis and their relationship to cardiorenal outcomes. Diabetes Obes Metabol 2022;24:1061–71.
[41] Rich NE, Oji S, Mufti AR, et al. Racial and ethnic disparities in nonalcoholic fatty liver disease prevalence, severity, and outcomes in the United States: a systematic review and meta-analysis. Clin Gastroenterol Hepatol : the official clinical practice journal of the American Gastroenterological Association 2018;16:198–210.e192.