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Research paper

T2DM/CKD genetic risk scores and the progression of diabetic kidney disease in T2DM subjects

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ABSTRACT

This study aimed at understanding the predictive potential of genetic risk scores (GRS) for diabetic kidney disease (DKD) progression in patients with type 2 diabetes mellitus (T2DM) and Major Cardiovascular Events (MCVE) and All-Cause Mortality (ACM) as secondary outcomes. We evaluated 30 T2DM and CKD GWAS-derived single nucleotide polymorphisms (SNPs) and their association with clinical outcomes in a central European cohort (n = 400 patients). Our univariate Cox analysis revealed significant associations of age, duration of diabetes, diastolic blood pressure, total cholesterol and eGFR with progression of DKD (all $P < 0.05$). However, no single SNP was conclusively associated with progression to DKD, with only *CERS2* and *SHROOM3* approaching statistical significance. While a single SNP was associated with MCVE – *WSF1* ($P = 0.029$), several variants were associated with ACM – specifically *CANCAS1*, *CERS2* and *C9* (all $P < 0.02$). Our GRS did not outperform classical clinical factors in predicting progression to DKD, MCVE or ACM. More precisely, we observed an increase only in the area under the curve (AUC) in the model combining genetic and clinical factors compared to the clinical model alone, with values of 0.582 (95 % CI 0.487–0.676) and 0.645 (95 % CI 0.556–0.735), respectively. However, this difference did not reach statistical significance ($P = 0.06$).

This study highlights the complexity of genetic predictors and their interplay with clinical factors in DKD progression. Despite the promise of personalised medicine through genetic markers, our findings suggest that current clinical factors remain paramount in the prediction of DKD. In conclusion, our results indicate that GWAS-derived GRSs for T2DM and CKD do not offer improved predictive ability over traditional clinical factors in the studied Czech T2DM population.

1. Introduction

Diabetic kidney disease (DKD) is a serious complication affecting 30–40 % of patients with diabetes (Gu, 2019). As the most common cause of kidney failure worldwide, DKD represents a significant health and socioeconomic burden. The clinical course of DKD is known to be highly variable, with up to 40 % of patients with type 2 diabetes (T2DM) developing DKD without prior or concomitant albuminuria or elevated albumin/creatinine ratio (Yamanouchi et al., 2020; Gonzalez Suarez et al., 2013). The pathophysiological and histopathological heterogeneity – as suggested by clinical and scarce renal biopsy data in T2DM (Di Vincenzo et al., 2020) – suggests that in addition to diabetic

Abbreviations: ACM, All cause mortality; CKD, Chronic kidney disease; CVD, Cardiovascular disease; DKD, Diabetic kidney disease; eGFR, estimated Glomerular filtration rate; ESRD, End-stage kidney disease; w/uGRS, weighted/unweighted Genetic risk score; GWAS, Genome wide association studies; MCVE, Major Cardiovascular event; PRS, Polygenic risk score; T2DM, Type 2 Diabetes mellitus; TG, Triacylglycerols; uACR, urinary Albumin/Creatinine ratio; UAE, Urinary Albumin excretion.

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nephropathy, other renal pathologies (such as hypertensive nephropathy, interstitial nephritis, etc.) may contribute to the final DKD phenotype. Some studies suggest that up to 50 % of DKD is actually non-diabetic chronic kidney disease (CKD) (Gonzalez Suarez et al., 2013). In addition, several recent studies show a lower risk of progression to DKD or death in patients with non-proteinuric DKD (Yamanouchi et al., 2020).

Identifying patients at higher risk of DKD/CKD early in the disease process and subsequently personalising antidiabetic, antihypertensive and renoprotective therapy would be of great value. Biomarkers are therefore needed to reliably identify patients at higher risk of developing DKD and its more severe course, and some of the published biomarkers may have diagnostic potential (e.g. uric acid, vitamin D, FGF 23, TNFR1, TNFR2 or CKD273 classifier score) (Gonzalez Suarez et al., 2013; Kalantar-Zadeh et al., 2021; Good et al., 2010).

DKD is partly genetically determined, but the genetic background is not fully understood despite extensive experimental efforts. Major pitfalls in studying the genetics of DKD have been identified: phenotypic heterogeneity, polygenic nature of genetic susceptibility, and incomparably smaller cohorts available for genome-wide association studies (GWAS) compared to T2DM or T1DM. The genetic architecture of DKD has also been studied and the results have recently been reviewed (Gu, 2019). Despite these complications, 41 SNPs have already been associated with DKD traits in diabetic populations (Sandholm and Groop, 2018).

The genetic structure of T2DM has effectively been investigated by means of numerous GWAS across different populations and consortia, revealing several hundreds of risk variants to date (Vujkovic et al., 2020; Xue et al., 2018). It is widely acknowledged that the greater the genetic susceptibility to T2DM in an individual, the more earlier disease manifestation and more rapid progression, thereby leading to the development of more severe complications, such as DKD. Although the contribution of each associated SNP to overall genetic risk seems to be low, the composite "genetic burden" expressed as a genetic risk score (GRS) could offer a practical method to quantify an individual's risk. For instance, a GRS composed of 65 SNPs has shown an association with a three-fold increase in T2DM development risk within a European Caucasian population (Talmud et al., 2015; Hubacek et al., 2023).

Alongside the endeavours to uncover the genetic basis of DKD, several GWAS in CKD patients from various ethnic backgrounds has revealed numerous variations linked to susceptibility to CKD (Cañadas-Garre et al., 2019), its progression (Parsa et al., 2017), and kidney function as a quantitative trait (Wuttke and Köttgen, 2016).

Our study aimed to examine whether increased genetic susceptibility, as indicated by a T2DM, CKD and combined GRS, contributes to a more aggressive and earlier onset of DKD. It is important to note that DKD is a multifaceted condition encompassing both diabetic and non-diabetic pathologies. We have considered the top 21 associated SNPs from established T2DM risk GRS (Talmud et al., 2015) and 9 SNPs associated with CKD/DKD (Table S1) or other kidney traits. We have then computed unweighted and weighted GRS to assess their possible association with DKD progression, major cardiovascular outcomes, and overall survival in patients with T2DM.

2. Material and methods

2.1. Study population and participants

The study included individuals of Caucasian descent who live in the southern Moravia region of the Czech Republic. These individuals were followed in Diabetes centres and event. Nephrology and dialysis outpatient clinics of the two university hospitals in Brno (St. Anne's and Brno-Bohunice hospitals). The participants had different stages of DKD baseline and were originally enrolled to be followed prospectively for the study of the natural history of diabetic complications between 2005 and 2013. The study design, inclusion/exclusion criteria and baseline study population characteristics were previously published (Pácal et al., 2011). The patient data had been updated in 2021 – 22, for some subjects enrolled originally DNA was no longer available. Therefore, current study sample comprised n = 400 unrelated T2DM patients (205 males and 195 females) whose DNA samples were of adequate quality for analysis. Table 1 displays the basic clinical characteristics of the subjects. The prospective data was collected until January 2022. The stage of DKD was determined by urinary albumin excretion (UAE) and estimated glomerular filtration rate (eGFR). The study population's baseline consisted of individuals with normoalbuminuria (n = 27, UAE < 30 mg/24 h, 8.8 %), persistent microalbuminuria (n = 92, UAE 30–300 mg/24 h, 27.1 %), and macroalbuminuria (n = 224, UAE > 300 mg/24 h, 51.1 %). The staging of DKD by (eGFRwas as follows: CKD 1 (n = 58, eGFR ≥ 90 ml/min per 1.73 m2, 16.7 %), CKD 2 (n = 68, eGFR 60–89 ml/min per 1.73 m2, 18 %), CKD 3 (n = 133; eGFR 30–59 ml/min per 1.73 m2), CKD 4 (n = 58; eGFR 15–29 ml/min per 1.73 m2) and CKD 5/end-stage renal disease (n = 49; eGFR < 15 ml/min per 1.73 m2 or ongoing haemodialysis). Both eGFR and UAE stage were measured at least every six months, with staging based on two consecutive values. The baseline structure of study sample defined by the Kidney Disease Improving Global Outcomes (KDIGO) categories shows Table 2. At baseline, 48 % of patients presented with retinopathy and 45.3 % with neuropathy. Treatment for diabetes in this cohort included both oral anti-diabetic drugs (62 % of subjects) and insulin (66 % of subjects). As renoprotective treatment, patients predominantly received angiotensin-converting enzyme inhibitors (60 %) and angiotensin II type 2 receptor blockers (41 %). Other antihypertensive drugs or diuretics were used in polypharmacotherapy. However, considering the historical nature of the cohort, no SGLT-2 inhibitors or GLP-1 analogues were lacking as antidiabetic treatment for the most part of the follow-up. The research was carried out following the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine, Masaryk University, Brno (No. 8/2006). Each patient has signed an informed consent for inclusion in the study.

2.2. Follow-up data

The study followed patients for a median of 54.6 months (IQR 27.8–91). The primary objective was to investigate DKD progression, defined as a transition from a baseline stage except ESRD based on the KDIGO "heat map" either by UAE or CKD stage (refer to Table 2). Additionally, two secondary objectives were evaluated: (i) incidence of major cardiovascular events (MCVE), including fatal or non-fatal myocardial infarction, stroke, limb amputation, or revascularization and (ii) all-cause mortality (ACM). For the purposes of analysing DKD progression, individuals with baseline ESRD (n = 58) were excluded as they could not progress.

2.3. Genotyping

Blood samples were collected from each participant at the beginning of the study. The DNA was isolated from peripheral blood leukocytes using the phenol–chloroform method and subsequently stored at −20 °C for analysis. The genotyping was performed using pre-designed TaqMan Genotyping assays (Life Technologies), following the manufacturer's recommended protocol on the LightCycler® 96 Instrument (Roche Applied Science, Mannheim, Germany). Further details on the IDs for specific assays used can be found in Table S1.

2.4. GRS construction

A total of five GRS were constructed, comprising both weighted (wGRS) and unweighted (uGRS) GRS, and the details are provided in Table S1. The unweighted GRS was created solely by summing the risk alleles (2 points for homozygotes carrying the risk allele, 1 for heterozygotes, and 0 for homozygotes carrying the non-effect allele). In this

Table 1
General characteristics of examined patients at the enrolment.

Parameter	CKD1	CKD2	CKD3	CKD4	CKD5	P
n	58	68	133	58	49	
Gender (female, %)	48.4	50	49.2	38.8	45.4	
Age	59.5 [51–67]	68 [60–74]	69 [63.5–77]	69 [62–75]	71 [62–77]	< 0.001
BMI (kg/m^2)	31.7 [28.4–38.1]	31 [27.7–33.9]	30.1 [26.7–33.2]	29.8 [27.02–33.96]	28.6 [25.1–31.07]	
SBP (mmHg)	150 [140–160]	145 [120–160]	147 [133–160]	140 [130–140]	142 [126–155]	ns
DBP (mmHg)	90 [80–95]	80 [80–90]	80 [72–90]	80 [70–80]	73 [66–81]	0.02
HbA1c (%)	7.6 [5.3–9.1]	6.2 [5.4–7.5]	7.6 [6.1–8.8]	6.25 [5.45–7.3]	6.4 [5.1–8]	ns
UREA (mmol/l)	5.7 [4.9–7.1]	7.8 [6.3–9.4]	11.8 [9–15.1]	19.4 [16.5–25.8]	23.9 [21–28]	< 0.001
Creatinine (μmol/l)	88 [82–101]	109 [92–128]	148 [128–181]	261 [209–311]	538 [462–653]	< 0.001
TC (mmol/l)	5.1 [4.4–6.1]	4.8 [3.9–5.7]	5 [4.3–6]	4.6 [3.9–5.3]	4.6 [3.8–5.6]	ns
TG (mmol/l)	2.3 [1.6–3.6]	1.7 [1.38–2.86]	2.0 [1.34–2.7]	2.0 [1.5–2.8]	2.1 [1.62–3.29]	ns
proteinuria (g/24 h)	0.29 [0.1–0.9]	0.2 [0.093–1.62]	0.6 [0.2–2.07]	1.3 [0.34–2.89]	0.7 [0.4–1.29]	0.01
GFR (ml/s)	1.8 [1.6–2.3]	1.2 [1.1–1.34]	0.8 [0.63–0.86]	0.4 [0.3–0.44]	0.2 [0.21–0.23]	< 0.001
Hypertension (%)	91.9	95.6	94.1	87	87.3	
Treatment	n = 37	n = 46	n = 98	n = 41	n = 37	
PAD (%)	78	70	67	54	35	
Insulin (%)	43	61	70	80	65	
ACEI (%)	70	63	60	44	65	
ARB (%)	30	33	54	37	38	

Data are expressed as median [IQ range] if not stated otherwise. SBP – systolic blood pressure, DBP – diastolic blood pressure, TC – total cholesterol, TG – triacylglycerol, GFR – estimated glomerular filtration rate, PAD – peroral antidiabetics, ARB – therapy with angiotensin II blockers, ACEI – therapy with ACE inhibitors.

Table 2
Baseline distribution of study participants according to KDIGO criteria.

			Persistent albuminuria categories		
			A1	A2	A3
			<30 mg/24h	30-300 mg/24h	>300 mg/24 h
GFR categories (ml/min per 1.73 m2)	G1	>90	17	15	26
	G2	60-89	9	21	37
	G3a	45-89	1	22	47
	G3b	30-44	0	21	41
	G4	15-29	0	13	45
	G5	<15	0	0	49

study, three uGRS were calculated: uGRS-21, uGRS-9, and a composite uGRS-30. The construction of the weighted GRS for SNPs linked to T2DM (wGRS-21) was based on the methodology previously published in (Ding et al., 2011). Briefly, we computed the logarithm of the OR for each risk allele, then summarised all the logarithmic OR values as per the number of risk alleles presented. All of these 21 SNPs associated with T2DM were chosen from the previous GWAS and according to our design of the study OR were also used form this original study (Talmud et al., 2015). Additionally, we calculated the Czech population-specific wGRS (wGRS-6) that was associated with SNPs linked to T2DM in our previous research (Hubacek et al., 2023). Further information regarding specific SNPs for each GRS can be found in Table S1. SNPs used for construction of uGRS-9 and uGRS-30 were chosen based on their association with either DKD/CKD phenotype or intermediate renal traits based on the literature review (details can be seen in Table 3).

To conduct time-to-event analysis, all 5 GRSs (as shown in Figure S1) were divided into quartiles. Additionally, the top decile of a GRS was compared against the remaining 90 %.

Table 3
SNPs used in construction of uGRS-9 and uGRS-30.

SNP	Nearest gene	Associated with	Source
rs3850625	CANCAS1	UACR	(Wuttke et al., 2019)
rs7805747	PRKAG2	CKD	(Köttgen et al., 2010 May)
rs267738	CERS2	GFR	(Köttgen et al., 2010 May)
rs347685	TFDP2	CKD	(Köttgen et al., 2010 May)
rs77924615	PDILT	GFR	(Stanzick et al., 2021)
rs113956264	RPL3L	GFR	(Stanzick et al., 2021)
rs700233	C9	GFR	(Zhao et al., 2022)
rs1801239	CUBN	UACR	(Teumer et al., 2016)
rs17319721	SHROOM3	CKD	(Pattaro et al., 2016 Jan)

2.5. Prediction of clinical model

There were total of six models, and their details are listed in Table 4. In brief, clinical parameters and SNPs associated in the univariate model were used to establish a multivariate model. Two different models,

Table 4
Multivariate Cox regression model for each of the followed endpoints.

DKD progression	Clinical model			Genetic model			
	RR	95 % CI	P value	RR	95 % CI	Calculated for	P value
Age	1.01	0.99–1.02	0.25	1	0.98–1.02	x	0.69
Duration of diabetes	1.00	0.98–1.02	0.63	1	0.98–1.03	x	0.43
TC	0.76	0.64–0.89	<0.01	0.76	0.63–0.91	x	<0.01
Proteinuria	1.16	1.1–1.22	<0.001	1.14	1.06–1.21	x	<0.001
eGFR	0.41	0.27–0.62	<0.001	0.46	0.27–0.62	x	<0.001
CERS2	x	x	x	2	0.46–8.71	CC vs GG	0.35
MCVE	Clinical model					Genetic model	
	RR	95 % CI	P value	RR	95 % CI	Calculated for	P value
Age	1.02	0.99–1.05	0.11	1.01	0.98–1.05	x	0.43
eGFR	0.47	0.25–0.85	0.01	0.54	0.27–1.01	x	0.06
TG	0.88	0.69–1.07	0.23	0.85	0.65–1.06	x	0.16
WSF1	x	x	x	2.54	1.28–5	GG vs GT	0.02
CERS2	x	x	x	1.7	0.22–12.95	CC vs GG	0.22
ACM	Clinical model					Genetic model	
	RR	95 % CI	P value	RR	95 % CI	Calculated for	P value
Age	1.03	1.01–1.06	<0.01	1.02	0.99–1.05	x	0.08
Duration of diabetes	1.02	0.99–1.04	0.053	1.02	0.99–1.05	x	0.06
TC	0.80	0.65–0.97	0.03	0.76	0.96–1.30	x	0.03
Proteinuria	1.09	1.03–1.15	<0.01	1.08	0.99–1.16	x	0.06
eGFR	0.23	0.12–0.42	<0.001	0.26	0.13–0.49	x	<0.001
CERS2	x	x	x	1.8	0.4–7.96	CC vs GG	0.76
ZMIZ1	x	x	x	1.38	0.6–3.17	AA vs GG	0.72
CANCAS1	x	x	x	5.01	1.73–14.5	GG vs AA	<0.01
C9	x	x	x	1.71	0.79–3.68	AA vs GG	0.22

TC – Total cholesterol, TG – Triacylglycerol, eGFR – estimated glomerular filtration rate.

namely the clinical model (which only considers clinical parameters associated in the univariate analysis) and the genetic model (which considers both clinical parameters and SNPs associated in the univariate analysis), were computed and compared for the primary and secondary outcomes.

2.6. Statistical analysis

Basic characteristics (absolute and relative frequencies, median and IQR, mean and standard deviation) were used for the description of studied variables. The relation between the two variables was described by the Spearman correlation coefficient. The Mann-Whitney test was applied for the comparison of GRSs between the two groups. The method ROC curves were used for the comparison of proposed models.

Software Statistica 14.0 was used for the log-rank test and for the construction of Kaplan-Meier curves. The Cox regression model was used to adjust the results of survival for selected variables using JMP15.2.0, SAS Institute Inc. 2019 software. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical and genetic parameters and the endpoints

The allele frequencies for all identified SNPs are presented in Table S1. An analysis of univariate Cox regression revealed a significant impact of age ($P < 0.01$), diabetes duration ($P < 0.01$), diastolic blood pressure ($P = 0.023$), total cholesterol ($P < 0.01$), and eGFR ($P < 0.01$) on DKD progression. Table 5 provides comprehensive details of the results. None of the SNPs were found to be statistically significant in terms of DKD progression. However, *CERS2* ($P = 0.054$) and *SHROOM3* ($P = 0.08$) were found to be close to statistical significance (Table 5). Multivariate Cox regression analysis revealed that age ($P = 0.04$), urea ($P < 0.01$), creatinine ($P = 0.014$), triacyclglycerols (TG) ($P = 0.03$), and eGFR ($P < 0.01$) were statistically significantly associated with MCVE. The only variant found to be significantly associated with MCVE was the SNP in *WSF1* ($P = 0.029$), and the SNP in *CERS2* had an almost statistically significant association ($P = 0.062$; Table 5).

There were significant statistical associations (all $P < 0.01$) between ACM and age, duration of diabetes, urea concentration, creatinine, total cholesterol (TC), proteinuria, and eGFR (Table 1). Additionally, there was an almost significant statistical association ($P = 0.069$) between ACM and diastolic blood pressure. Three of the studied SNPs demonstrated statistical significance in the analysis. The specific SNPs which proved significant were *CANCAS1* ($P < 0.01$), *CERS2* ($P = 0.017$), and *C9* ($P = 0.013$). Additionally, the *ZMIZ1* gene displayed marginal statistical significance ($P = 0.09$). Further details regarding all three traits and SNPs are available in Table 5.

3.2. GRS associations

The cumulative incidence of DKD progression, MCVE, and ACM were observed to be 53.5 %, 33.5 %, and 44.9 %, respectively. There were no significant differences found between defined groups (i.e. GRS quartiles or top decile vs. the rest) in any of the studied endpoints through time-to-event analysis. All measured $P > 0.05$ based on the log-rank test.

Correlations were also tested between GRS and age, diabetes duration, creatinine, and GFR, but no significant correlations were found (all $P > 0.05$, Spearman). However, there was a negative correlation between uGRS-30 and HbA1c ($r = -0.18$, $P = 0.044$). More details are in Table S2.

Comparison of GRSs between a group of individuals with DKD who progressed and a group who did not progress showed a slightly higher wGRS-21 in progressors than in non-progressors, as determined by the Mann-Whitney test ($P = 0.02$). There were no differences in other scores between the two groups.

3.3. The predictive power of genetic model vs. Clinical model

In the second phase of the study, variables with significant effects identified by the univariate analysis were incorporated into the multivariate model. The analysis determined that genetic variant *CANCAS1*

Table 5
Univariate Cox regression analysis.

Variable	DKD progression		
	RR	95 % CI	P
Age	1.02	1.01–1.04	0.0001
Diabetes duration	1.03	1.01–1.04	0.0004
DKD stage	1.62	1.27–2.09	0.0001
DBP	0.97	0.96–0.99	0.0237
Total cholesterol	0.82	0.7–0.95	0.008
Urea	1.12	1.09–1.14	0.0001
Creatinine	1.01	1.008–1.012	0.0001
Proteinuria	1.07	1.03–1.11	0.0001
eGFR	0.35	0.25–0.49	0.0001
CERS2	0.73	0.54–1.00	0.0539
SHROOM3	0.8	0.63–1.02	0.0826
Variable	**MCVE**		
	RR	95% CI	P
Age	1.02	1.00–1.04	0.0437
DKD stage	1.46	1.09–1.96	0.0101
Urea	1.06	1.03–1.09	0.0001
TG	0.82	0.65–0.98	0.0337
Creatinine	1.00	1.000–1.003	0.0146
eGFR	0.32	0.18–0.54	0.0001
WSF1	1.72	1.12–2.68	0.029
CERS2	0.65	0.43–1.02	0.062
Variable	**ACM**		
	RR	95% CI	P
Age	1.04	1.02–1.06	0.0001
Diabetes duration	1.04	1.02–1.06	0.0001
DKD stage	2.64	2.1–3.35	0.0001
DBP	0.98	0.96–1.00	0.0696
Urea	1.09	1.07–1.1	0.0001
Total cholesterol	0.78	0.67–0.91	0.0017
Proteinuria	1.07	1.02–1.11	0.0034
eGFR	0.2	0.12–0,32	0.0001
ZMIZ1	0.82	0.66–1.03	0.0902
CANCAS	1.53	1.11–2.01	0.0097
CERS2	0.67	0.49–0.93	0.0177
C9	1.35	0.06–1.73	0.013

Data represented if P < 0.1, TG – triacyglycerol, DBP – diastolic blood pressure, eGFR – estimated glomerular filtration rate, DKD stage – Diabetic kidney disease stage.

(P = 0.003, RR = 5.01, 95 % CI 1.73–14.5) had an impact on ACM, whereas *WSF1* (P = 0.007, RR = 2.53, 95 % CI 1.28–5.00) had an effect on MCVE (Table 4). However, we were unable to confirm any association between *CERS2* and either DKD or MCVE, as well as between ACM and *ZMIZ1*, *CERS2*, and *C9* in the multivariate analysis (Table 4).

Furthermore, there were no discrepancies between the clinical model and the genetic model in any of the endpoints studied. The AUC curve exhibited a minor increase (P = 0.066) in the genotypic MCVE model in contrast to the clinical model, registering respective scores of 0.582 (95 % CI 0.487–0.676) and 0.645 (95 % CI 0.556–0.735). AUC curves can be seen in Fig. 1.

Not all genetic or clinical parameters that were associated with univariate analysis showed an association with multivariate analysis. Accordingly, we created another multivariate model that excluded non-associated parameters. However, this did not improve the predictive ability of our genetic models. (Data not shown).

Finally, we investigated whether SNPs linked to kidney traits in prior research (see Table S1 and Table 3) and incorporated into our GRS are linked to kidney traits in the Czech population. Our findings indicate no significant association between these 9 SNPs and GFR, creatinine or proteinuria in our cohort.

4. Discussion

Unlike circulating biomarkers of DKD, whose levels can be influenced by various confounders such as sex, age, comorbidities, or pharmacotherapy, genetic markers remain stable and can be determined at any point in an individual's lifespan. Identifying those at risk based on genetic markers could improve their future quality of life and decrease the burden on medical care. In this study, we developed wGRS and uGRS using SNPs linked to T2DM or kidney traits to evaluate its predictive power the progression of DKD among T2DM patients in a racially homogeneous Central European community. The study indicated a considerable correlation between ACM and *CANCAS1* (P = 0.01), *CERS2* (P = 0.017), and *C9* (P = 0.01). The SNPs investigated in this study, which were linked with ACM, have been previously found to be associated with kidney traits in literature. However, we were unable to replicate these findings (data not shown). The SNPs associated with coronary artery plaque calcification and myocardial infarction were utilised to calculate both weighted and unweighted GRS in patients from the Diabetes Heart Study (Adams et al., 2014). The authors discovered that uGRS, which contained SNPs linked with CAC, and wGRS, made up of SNPs associated with both CAC and MI risks, were associated with CVD and MI, respectively. Another study which employed GRS associated with CVD, was the Look AHEAD trial (Look AHEAD Research Group, 2015). GRS originated from 153 SNPs related to coronary artery disease. Participants placed in the highest quartile of GRS demonstrated a 51 % higher incidence of CVD compared to those in the lowest quartile.

A recent study conducted in Egypt indicated a significant decrease in *WSF1* gene expression among patients diagnosed with both T2DM and DKD compared to those with T2DM without complications. The peripheral blood expression of *WSF1* showed a significant negative correlation with HbA1c levels (Sharaf et al., 2018). HbA1c as a well-established biomarker assessing glycaemia profile over the several weeks and thus clinical compensation in both T1DM and T2DM patients was firmly associated with a higher risk of comorbidities in these patients. Therefore, it may appear unexpected to find a negative correlation between wGRS – also supposedly indicating a higher risk – and HbA1c in our study. The interpretation of this finding is purely speculative at this stage, yet, some hypotheses can be proposed. For example, patients with a higher GRS manifesting diabetes earlier may be given earlier treatment and this may lead to a longer period of satisfactory compensation and subsequently to lower HbA1c.

A comprehensive study of 2755 Asian patients with T2DM was conducted to identify predictors of CKD by examining 25 clinical variables and 35 genetic variants (Jiang et al., 2016). The authors identified three novel predictors of CKD in T2DM patients: rs478333 in *G6PC2* and rs7754840 and rs7756992 in *CDKAL1*. However, DKD is observed more frequently among Asians than among Caucasians (60 % versus 30–40 %, respectively).

Our study did not associate any individual SNP with the progression of DKD, albeit the gene *SHROOM3* showed nearly significant statistical association. Secondly, the *CERS2* gene demonstrated near significance (P = 0.053), with the same SNP in this gene being linked to accelerated albuminuria progression in diabetic patients (Shiffman et al., 2014). After analysing secondary outcomes, the MCVE showed that the *WSF1* variant is significantly associated (P = 0.02). Based on the literature available, we believe that we are the first to establish the association of this gene with MCVE in the Caucasian population. We found that the heterozygote is associated with the disease compared to both homozygotes (as shown in Table 4). This phenomenon may be caused by the law of small numbers since our study group comprised only 400 patients.

Our outcomes can be summarised as follows: GRSs (considered as quartiles or top ten percent versus the other percentile) showed no association with examined outcomes except wGRS-21 in relation to DKD progression. Although the median time to progression was comparable across quartiles/deciles of T2DM subgroups for wGRS-21 (according to time-to-event analysis), the values of wGRS-21 were significantly disparate between DKD progressors and non-progressors. Identifying individuals with a higher likelihood of progression could be a useful tool in the future to empower patients and by more aggressive management

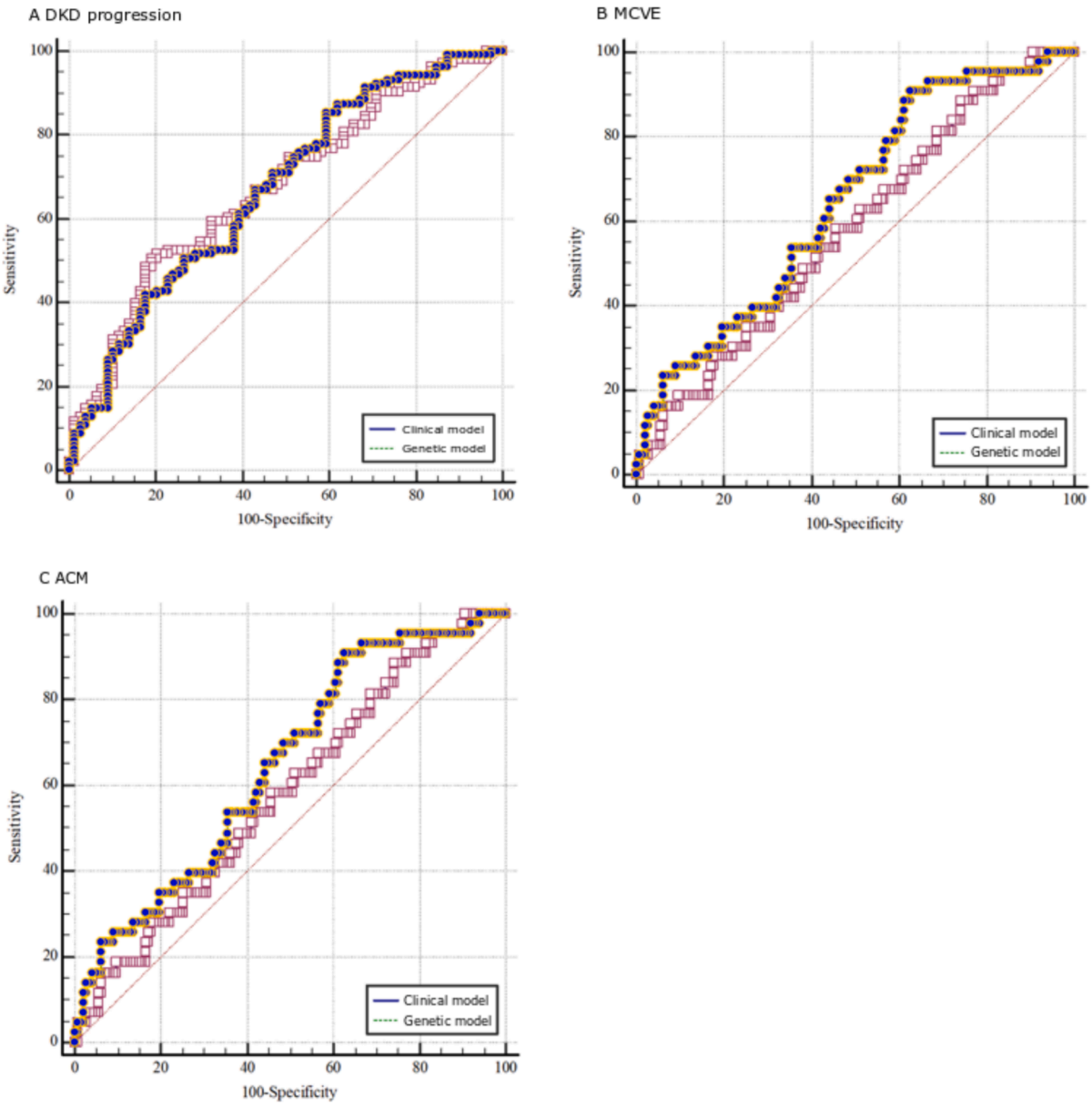

Fig. 1. Comparison of AUC curves in different models. A is comparison of Clinical and genetic model in DKD endpoint, B is comparison of clinical and genetic model in MCVE endpoint and C is comparison in clinical and genetic model in ACM endpoint.

to prevent progression. This strategy may significantly improve the patient's quality of life and reduce healthcare costs associated with dialysis or kidney transplantation due to DKD.

Our study is consistent with research conducted on French individuals with T2DM and T1DM (Barbieux et al., 2019). The authors utilised a GRS comprising 18 SNPs, with only one SNP in the *SHROOM3* gene overlapping with our study (unless otherwise indicated, there were no overlaps with our investigation). The SNPs were correlated with renal function or CKD stage 5. The authors did not detect a link between GRS and renal outcomes, defined as serum creatinine doubling or end-stage renal disease. Another study conducted on Italian patients with T2DM examined the significance of renal and cardiovascular (CV) GRS on kidney function (Zusi et al., 2018). The renal GRS included 39 SNPs, while the CV GRS was calculated from 42 SNPs. The authors discovered a correlation between the renal GRS and a decrease in eGFR. In a separate investigation, a weighted GRS made up of 53 SNPs (17 SNPs coinciding with (Zusi et al., 2018) was created to investigate if a higher GRS can forecast progression to stage 3 CKD, without any influence from prevalent clinical risk factors among the Caucasian population (Ma et al., 2017). The authors found that an elevated GRS could predict the progression to stage 3 CKD, however, it lacked significant prediction enhancement when compared to widespread clinical risk factors. The study employed the UK Biobank sample, containing 452,000 participants, to analyse the GRS associated with GFR. Findings showed that a lower GRS (connoting lower eGFR) composed of 147 SNPs, a mere few of which overlapped with those used in previous studies (Zusi et al., 2018; Ma et al., 2017), was significantly connected to an increased risk of hypertensive disease, chronic renal failure, acute kidney injury, and glomerular disease within this cohort (Wuttke and Köttgen, 2016). Another genome-wide polygenic risk score (PRS) consisting of eight SNP variants associated with CKD was examined in a Japanese population by Fujii et al. (Fujii et al., 2019). The study authors observed that an increase in GRS was linked to the presence of CKD. Xu and colleagues (Xu et al., 2016) employed a novel methodology by generating a GRS from T2DM risk variants and examining its causal impact on eGFR and urinary albumin/creatinine ratio (uACR) in an East Asian (Chinese) cohort.

Their application of Mendelian randomization revealed a causal association between GRS and decreased eGFR. Additionally, two GRS subgroups were generated based on loci associated with insulin secretion and insulin resistance. Both subgroups exhibited an association with decline in eGFR and uACR. Our study identified three specific SNPs in the *IGF2BP2*, *CDKN2A/B* and *TCF7L2* genes that overlapped with these findings. Another study conducted on the Han Chinese population found an increased risk of DKD by 1.22 (95 % CI 1.15–1.29) per risk allele after creating a wGRS from 7 SNPs. The researchers compared the predictive abilities of three distinct models: the clinical model, the genetic model, and the combined clinical and genetic model. The areas under the receiver operating characteristic (AUROC) curves were found to be 0.75 (95 % CI 0.72–0.78), 0.64 (95 % CI 0.60–0.68) and 0.78 (95 % CI 0.75–0.81) with a P-value of 0.002. Hence, it can be inferred that the utilization of genetic markers increased the predictability of the model.

In contrast, a number of studies failed to establish a link between the GRS and the prediction of CKD/DKD progression or its development (O'Seaghdha et al., 2012; Thio et al., 2018), or to enhance the predictive power of genetic plus clinical model when compared to clinical model (Ma et al., 2017). Variations in the results of genetic studies of diseases such as T2DM or its renal complications can be attributed to several key factors. These include the selection of different SNPs to construct GRSs, variations in the way diseases are defined (phenotype definitions), and ethnic differences that affect the prevalence of particular SNPs. It is important to note that these factors can contribute to differences in study outcomes and should be taken into consideration when interpreting results. Furthermore, it is important to consider the potential impact of gene-environment interactions and lifestyle differences on disease manifestation and progression within different study populations. Additionally, there may be discrepancies regarding the comparative efficacy of clinical versus genetic predictive models, with some studies indicating added value in genetic information while others do not.

Two limitations of the study must be acknowledged. Firstly, studies typically select different sets of SNPs based on the authors' preferences, as well as different quantities of SNPs for the construction of GRS. Additionally, it is recognised that SNPs associated with T2DM, or its complications vary across various ethnic groups, leading to difficulties when comparing different studies. Secondly, the study sample size is relatively small, yet, with reasonable follow-up and comprehensive clinical data available. Therefore, it is not possible to exclude a type II error in our cohort, particularly in SNPs with a small effect size that require a large group of patients to establish an association.

The concept of utilizing personalised medicine to tailor care and prevention to each individual's needs is becoming increasingly realistic with our ongoing understanding of genetics of complex diseases. However, to correctly predict a given phenotype progression, appropriate and well-characterised SNPs need to be selected. These are likely to vary between different ethnic groups.

Our findings, supported by other literature, indicate that classical clinical factors currently possess comparable or superior predictive power for DKD prediction in comparison to GRS. Therefore, in the future, emphasis should be placed on selecting SNPs which can enhance this genetic predictive capacity, hence enabling personalised medicine to evolve as a field. This will improve patient care while simultaneously reducing healthcare costs. A recent study has demonstrated that genetic testing is a more cost-effective and efficient method for quality-adjusted life-year assessment than traditional clinical screening (Guinan et al., 2021). With technological advancements in genotyping and greater access to well-phenotyped DKD cohorts, we may be able to more accurately identify pathogenic loci in the future, leading us to better understand the genetic architecture of DKD.

In conclusion, our study demonstrated that currently there was no significant increase in the predictive potential of the GRS based on GWAS-derived risk variants for T2DM and CKD when compared to classical clinical factors, in Czech patients with T2DM.

Credit authorship contribution statement

David Galuška: Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Lukáš Pácal:** Writing – review & editing, Project administration, Methodology, Formal analysis, Conceptualization. **Katarína Chalásová:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Petra Divácká:** Writing – review & editing, Validation, Resources, Data curation, Conceptualization. **Jitka Řehořová:** Writing – review & editing, Validation, Resources, Data curation, Conceptualization. **Jan Svojanovský:** Writing – review & editing, Validation, Resources, Data curation, Conceptualization. **Jaroslav A. Hubáček:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Věra Lánská:** Writing – review & editing, Validation, Formal analysis. **Kateřina Kaňková:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2024.148724.

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