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Риск развития хронической болезни почек у больных сахарным диабетом 2 типа детерминирован полиморфизмом генов *NOS3, APOB, KCNJ11, TCF7L2*

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Генетическая предрасположенность является одним из важных факторов развития хронических диабетических осложнений.
Цель. *Изучить ассоциацию полиморфных маркеров (ПМ) комплекса генов-кандидатов, кодирующих основные медиаторы поражения почек, с развитием хронической болезни почек (ХБП) у пациентов с сахарным диабетом 2 типа (СД2).*
Материалы и методы. *В исследование включено 435 пациентов с СД2 по принципу «случай–контроль». Первая группа (n=253) формировалась при помощи неперекрывающихся критериев отбора: пациенты с ХБП при длительности СД2 менее 5 лет («ХБП+», n=78) и пациенты без ХБП с СД2 более 10 лет («ХБП-», n=175). В этой группе исследовали ПМ I/D гена ACE, ecNOS4a/4b гена NOS3, I/D гена APOB, e2/e3/e4 гена APOE. Во 2-й группе (n=182) пациенты были распределены на подгруппы «ХБП+» и «ХБП-» (n=38/144) вне зависимости от длительности СД, исследовали ПМ pro12ala гена PPARG2, rs5219 гена KCNG11, rs12255372 гена TCF7L2, rs13266634 гена SLC30A8. Статистический анализ распределения частот аллелей и генотипов проводили с использованием таблиц сопряженности и критерия χ^2, $p<0,05$.*
Результаты. *Достоверную ассоциацию с развитием осложнения показали 4 гена. Ген NOS3 (эндотелиальной синтетазы оксида азота): носительство аллеля 4a и генотипа 4a/4a гена NOS3 повышают риск развития ХБП в 2 раза (OR=2,26/9,88 соответственно), аллель 4b и генотип 4b/4b гена NOS3 являются защитными (OR=0,44/0,45 соответственно). Ген APOB (аполипопротеина B): носительство генотипа DD гена APOB имеет протективное значение (OR=0,20, 95% ДИ 0,05–0,88). Ген TCF7L2 (транскрипционного фактора 7, подобного фактору 2): носительство генотипа TT предрасполагает к развитию ХБП (OR=3,03, 95% ДИ 1,07–8,58). Ген KCNJ11 (субъединицы Kir6.2 АТФ-зависимого калиевого канала): носительство генотипа AA повышает риск развития ХБП (OR=2,25, 95% ДИ 1,02–4,97), носительство аллеля G защищает от развития ХБП (OR=0,57, 95% ДИ 0,34–0,96).*
Заключение. *В результате исследования установлено, что развитие ХБП при СД2 генетически детерминировано. Выявлена достоверная ассоциация риска ХБП с генами, кодирующими факторы эндотелия (NOS3), факторы липидного обмена (APOB) и факторы секреции инсулина (KCNJ11, TCF7L2), продукты экспрессии которых участвуют в основных патогенетических механизмах поражения почек при СД.*
Ключевые слова*: сахарный диабет; хроническая болезнь почек; генетическая предрасположенность; NOS3; APOB; TCF7L2; KCNJ11*

Risk of chronic kidney disease in type 2 diabetes determined by polymorphisms in *NOS3, APOB, KCNJ11, TCF7L2* genes as compound effect of risk genotypes combination

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Genetic susceptibility plays an important role in the risk of developing chronic complications in patients with type 2 diabetes mellitus (T2DM).
Aims. *In this study, we evaluated the possible association of the polymorphic variants that encode key renal damage mediators (endothelial dysfunction, lipid metabolism and insulin secretion/sensitivity) with the risk of chronic kidney disease (CKD) in patients with T2DM.*
Materials and Methods. *We enrolled 435 patients with T2DM using case-control study design. In 253 patients, we used non-overlapping criteria to form groups with/without CKD (defined as GFR<60 ml/min/1.73 m^2) according to the duration of T2DM (≤5 years/≥10 years) (n=75 and 178, respectively) and analysed the following 4 polymorphic markers: I/D in ACE, ecNOS4a/4b in NOS3, I/D in APOB and e2/e3/e4 in APOE genes. We then divided 182 patients in groups with/without CKD (n=38 and 144, respectively) regardless of the duration of diabetes and studied*

pro12ala in PPARG2, rs5219 in KCNJ11, rs12255372 in TCF7L2 and rs13266634 in SLC30A8 genes. Statistical analysis was performed using the χ^2 test, and data were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Values of p <0.05 indicated statistical significance.
Results. *Four genes were found to have a significant association with CKD occurrence. For the eNOS3 the allele 4a and 4a/4a genotype was associated with a twofold CKD risk (OR=2.2/9.88) and the allele 4b and 4b/4b polymorphism were protective regarding CKD development (OR=0.44/0.45). For APOB I/D, the genotype DD was associated with lower risk of CKD [OR for DD=0.2 (95% CI: 0.05–0.88)]. In the second group, genotype TT of TCF7L2 predisposed to CKD (OR=3.03, 95% CI: 1.07–8.58). For KCNJ11 group genotype AA predisposed to CKD (OR=2.25, 95% CI: 1.02–4.97) compared to the allele G (OR=0,57, 95% CI: 0.34–0.96).*
Conclusions. In conclusion, our findings indicate a significant role of functional genetic variants associated with genes of endothelial factors (NOS3), lipid metabolism (APOB), and insulin secretion factors (KCNJ11, TCF7L2) in modulating the risk of CKD and their significant involvement in the mechanism of kidney damage in patients with T2DM.
Keywords: *diabetes mellitus; genes; CKD; NOS3; APOB; TCF7L2; KCNJ11*

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Chronic kidney disease (CKD) is a pathology that, according to its growth in prevalence, is becoming a non-infectious epidemic along with such diseases as diabetes mellitus (DM) and obesity. CKD develops in 13–15% of individuals in the general population and much more frequently, up to 40–50%, in at-risk groups, such as patients with type 2 diabetes mellitus (T2DM) [1]. According to the International Diabetes Federation estimates, the number of diabetic patients in the world will increase to 552 million people by 2030, and 90% of these patients will have T2DM. The severity of T2DM is due to generalized vascular system diseases including the development of multiple micro- (e.g., nephropathy and retinopathy) and macrovascular (coronary heart disease (CHD) and coronary and peripheral atherosclerosis) complications. The incidence and development rate of vascular complications, in addition to modifiable factors (e.g., hyperglycaemia, arterial hypertension (AH) and dyslipidaemia), depend on the individual genetic features that characterize the greater or lesser sensitivity of an individual to the damaging effects of pathological factors in DM.

Diabetic nephropathy (DN) is one of the most dangerous complications of DM , it leads to a progressive decrease in the filtration function of the kidneys, and results in chronic renal failure (uraemia). Increased urinary protein excretion and microalbuminuria (MAU) followed by proteinuria (PU) are the classic signs of DN. The characteristic feature of renal disease in T2DM patients is the heterogeneity of renal dysfunction in this disease; this heterogeneity makes it nearly impossible to differentiate classical DM based on evaluations of protein excretion as can be performed in type 1 diabetes mellitus (T1DM) [2]. The term CKD was coined for the diagnosis of renal disease. CKD is a generalized trans-nosological concept and allows for evaluations of the presence and severity of renal disease regardless of the cause of injury. CKD is understood as the presence of one or several laboratory, structural or functional signs of kidney disorder lasting ≥3 months or an isolated decrease in glomerular filtration rate (GFR) to <60 ml/min/1.73 m^2. In the present our study, we were guided by the universally accepted classification of CKD and defined its presence as a stable (more than 3 months) decrease in GFR to <60 ml/min/1.73 m^2 [3].

Chronic hyperglycaemia is the main cause of all vascular complications of DM, including CKD. However, in some patients, kidney disease can develop and rapidly evolve despite satisfactory glycaemic control, which is indicative of the effect of non-glycaemic mechanisms. The relationships between the level of proteinuria, the degree of AH and the severity of glomerular sclerosis have been shown [4]. The first studies on the possibility of familial inheritance of diabetic renal disease were published in 1989 [5]. The identification of this group of patients was suggestive of the significant participation of genetic factors in the development of the DN and CKD.

CKD is the second most common cause of mortality in T2DM patients following cardiovascular disease. Control of blood glucose level and blood pressure using drugs that block the renin-angiotensin system (RAS) can slow the progression but cannot prevent the development of the disease. The study of genetic predisposition to CKD is of special significance from the perspective of the prediction and identification of risk groups in the preclinical stage when the pathological changes are potentially reversible.

The modern strategy of research into genetic predispositions to multifactorial diseases, such as vascular complications of DM, is based the study of polymorphic markers of candidate genes; i.e., genes whose expression products are involved in pathogenesis of this disease. A gene polymorphic marker (PM) is a variable region of DNA that is associated with a certain phenotypic trait (e.g., hypertension). The association of a genetic marker with a disease is understood to be reflected by a significant difference in the distributions of the frequencies of allele or paired set of alleles (genotype) between individuals with and without the pathology.

Objective

the objective of our work was to study the distributions of allele and genotype frequencies of the complex of polymorphic markers of candidate genes that are related to renal disease *(*the *I/D* polymorphism of the *ACE* gene, *ecNOS4a/4b* polymorphism of the *NOS3* gene, *I/D* polymorphism of the *APOB* gene, *ε2/ε3/ε4* polymorphism of the *APOE* gene, *pro12ala* polymorphism of the *PPARG2* gene, *rs5219* polymorphism of the *KCNJ11* gene, *rs12255372* polymorphism of the *TCF7L2* gene and *rs13266634* polymorphism of the *SLC30A8* gene*)* in T2DM patients with and without CKD and to evaluate the utility of studying these markers to predict renal injury in T2DM patients.

Table 1

Primer sequences and characteristics of the amplifications of the polymorphic regions of the *ACE, NOS3, APOB, APOE, KCNJ11, TCF7L2, PPARG2* and *SLC30A8* genes

Polymorphic markers	Forward and reverse primers (5' – 3')	$MgCl_2$, mM	Annealing, °C
KCNJ11 rs5219	TGCAGTTGCCTTTCTTGGACACAA GGTGGGGAGTTATCTCAGAAGTGAGGC	1	62
TCF7L2 rs12255372	CAGTTTACACATAAGGATGT CCTGAGTGATTATCAGAATA	2	48
PPARG2 pro12ala	TGCCCAAATAAGCTTTC GCAATGCATTAGGCACTA	1	58
SLC30A8 rs13266634	CAAACGTGGCTTCCTCTGA GTGAGTGAGTGCATCGTAAA	2	58
NOS3 (ecNOS34a/4b)	AGGCCCTATGGTAGTGCCTTT TCTCTTAGTGCTGTGGTCAC	1.5	53
APOB (I/D)	CAGCTGGCGATGGACCCGCCGA ACCGGCCCTGGCGCCCGCCAGCA	1.0	70
APOE (ε2/ε3 ε4)	ACGCGGGCACGGCTGTCCAA TCGCGGATGGCGCTGAGGC	2.0	66
ACE (I/D)	CTGGAGACCACTCCCATCCTTTCT GATGTGGCCATCACATTCGTCAGAT	1.5	62

Materials and methods:

the study included 435 Russian T2DM patients who were selected based on the "case-control" principle. The population was ethnically homogeneous. The first group (n = 253) was formed using the following non-overlapping selection criteria: patients with histories of CKD and T2DM for less than 5 years ("CKD+", n = 78); and patients without histories of CKD and T2DM for more than 10 years ("CKD–", n = 175). This group was used to study the *I/D* PM of the *ACE* gene, the *ecNOS4a/4b* PM of the *NOS3* gene, the *I/D* PM of the *APOB* gene and the *e2/e3/e4* PM of the *APOE* gene. The 2nd group of patients (n = 182) were divided into "CKD+" and "CKD–" subgroups (n = 38 and n = 144, respectively) independent of DM. These patients were examined for the *pro12ala* PM of the *PPARG2* gene, the *rs5219* PM of the *KCNG11* gene, the *rs12255372* PM of the *TCF7L2* gene and the *rs13266634* PM of the *SLC30A8* gene. This approach to group formation utilizing the "polar" phenotypes was employed used because CKD, particularly in T2DM, is a multifactorial disease, and we sought to reduce the masking effect of non-genetic risk factors of which the duration of hyperglycaemia is the most significant. The presence of CKD was defined as a persistent decrease in GFR to <60 ml/min/1.73 m^2 as calculated using the standard MDRD formula.

All patients provided informed consent. The study was approved by the local ethics committee of the Endocrinology Research Centre.

Allele identification was performed with the polymerase chain reaction (PCR) method. Genomic DNA was separated from the whole blood of the patients by phenol-chloroform extraction after incubating the blood samples with proteinase K in the presence of 0.1% sodium dodecyl sulphate. Thermostable Taq DNA polymerase purchased from ZAO Dialat (Moscow) was used. The oligonucleotide primers were synthesized by ZAO Evrogen (Moscow). Amplifications with known primer conditions were performed using real-time PCR and thermal cycling. The primer annealing temperature

Table 2

General characteristics of the groups with and without CKD

	Group 1 n = 253)			Group 2 n = 182)		
Clinical parameters	"CKD+" n = 78)	"CKD–" n = 175)	p	"CKD+" n = 78)	"CKD–" n = 175)	p
Gender (m/f)	35/43	39/136	p<0.05	12/26	60/84	N/D
Age, years	62.8±8.5	58.8±7.87	N/D	64.6±8.3	57.7±8.1	N/D
Duration of T2DM, years	5±0.6	12.5±2.7	design	15.5±9.65	10.8±6.3	p<0.05
HbA_{1c},%	7.8 ±1,8	8.6±1.9	p<0.05	8.0±1.92	9.1±1.92	p<0.05
Cholesterol, mmol/l	5.5 ± 1.8	5.2 ±3.1	N/D	5.5±1.26	5.4±1.42	N/D
Triglycerides mmol/l	2.7±1.8	1.9±1.2	p<0.05	2.0±0.89	2.2±1.9	N/D
SBP, mm Hg	147±20.4	144±18.4	N/D	146.7±15.9	137.1±16	p<0.05
DBP, mm Hg	79.4±12.4	88.9±11.2	p<0.05	81.8±10.8	81±10.2	N/D
BMI, kg/m	30.6 ± 5.3	32±6.7	N/D	32.7±7.66	31.6±6.1	N/D
GFR, mL/min/1.73 m^2	20.1 ±20.2	96.1 ±21.9	design	40±19.1	87.3±17.9	Design
Retinopathy,%	85	37	p<0.05	74	51	p<0.05
MAU, mg/l	3173 ±4797	36.5 ±69.68	p<0.05	103.5±44.9	17.5±49.7	p<0.05

Note: The data are presented as the "M ± the SD"; SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, GFR – glomerular filtration rate, MAU – microalbuminuria (in the morning urine portion), N/D – no significant difference.

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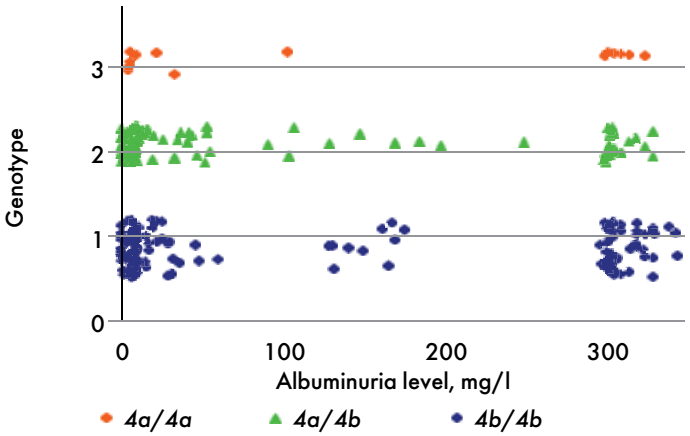

Fig. 1 Distribution of the NOS3 genotypes according to albuminuria level.

was varied depending on the locus to be amplified. The PCR conditions and primer sequences for the amplifications of the loci under study are shown in Table 1.

The amplified alleles of the polymorphic loci were identified after electrophoretic separation in 12% polyacrylamide gel or 2% agarose gel and subsequent staining with ethidium bromide. The observed genotype distributions were assessed for deviation from Hardy-Weinberg equilibrium.

The statistical analyses of the distributions of allele and genotype frequencies were performed using contingency tables and chi-square tests (χ^2). The clinical parameters were evaluated using Student's t test and the χ^2 method with Yates' correction. The differences were considered to be reliable at $p < 0.05$. The risk of developing CKD was calculated using the Clopper-Pearson method.

Results

The clinical characteristics of the patients are shown in Table 2. All patients were matched for age, sex and BMI. A significantly lower HbA_{1c} level was observed in the "CKD+" groups compared to the patients without CKD, which apparently reflected the potentially high incidence of hypoglycaemia in patients with lower filtration rates rather than improved carbohydrate metabolism control. Higher levels of albuminuria, triglycerides and systolic blood pressure (which are the major non-glycaemic risk factors for CKD progression) and a higher incidence of retinopathy as a result of combined end-organ damage were observed in the CKD patients.

The distributions of genotype frequencies in the "CKD–" population were consistent with the Hardy-Weinberg equilibrium for all polymorphisms. Significant associations with the development of CKD were found for markers of 4 of the genes.

***ecNOS4a/4b* marker of the endothelial nitric oxide synthase (*NOS3*) gene**: The *4a* allele and *4a/4a* genotype were significantly more frequent in the "CKD+" patients and had predisposing influences on the risk of developing CKD follows: *OR*= 2.26, 95% CI 1.45–3.54; and *OR*= 9.88, 95% CI 2.05–47.72, respectively. In contrast, the *4b* allele and *4b/4b* genotype had protective values as follows: *OR*= 0.44, 95% CI 0.29–0.69; and *OR*= 0.45, 95% CI 0.26–0.77, respectively (Table 3).

We also analysed the distribution of the genotypes of the *ecNOS4a/4b* marker of the *NOS3* gene according to albuminuria level. The data are shown in Fig. 1. No significant associations were detected, which might be indicative of different genetic determinants of the basic clinical markers of renal disease, i.e., GFR and albuminuria.

***I/D* marker of the *APOB* gene (apolipoprotein B):** The frequency of the *DD* genotype of the *APOB* gene in the CKD patients was significantly lower than that in the controls (2.7% vs. 12%, respectively, *p*= 0.02). According to a dominant inheritance model, the *DD* genotype of the *APOB* gene was found to play a protective role *(OR*= 0.20, 95% CI 0.05– 0.88); however, in the absence of this genotype, the risk of developing CKD increased fivefold compared to the *DD* genotype (p-value = 0.02, *OR* = 5.0, CI 95% 1.14–21.98). These data are shown in Table 3.

***rs5219* marker of the *KCNJ11* gene:** The *A* allele was significantly more common in the CKD patients, while the *G* allele was more common in the patients without CKD. A significant association of this marker with the development of CKD was revealed at the significance level of *p*= 0.03. The *A*

Table 3

Distribution of alleles and genotypes of the ecNO4a/4b marker of the NOS3 gene and the I/D marker of the APOB gene in patients with and without CKD

Alleles/genotypes	CKD+ (n = 78)	CKD– (n= 175)	χ^2	p	OR	95% CI
ecNOS4a/4b	Multiplicative inheritance model					
4a allele	47 (30.1%)	56 (16%)	12.43	0.0004	2.26	1.45–3.54
4b allele	109 (69.9 %)	294 (84%)	(df = 1)		0.44	0.29–0.69
General inheritance model						
4a/4a	8 (10.3%)	2 (1.1%)	16.1	0.00032	9.88	2.05–47.72
4a/4b	31 (39.7%)	52 (29.7%)	(df=2)		1.56	0.89–2.72
4b/4b	39 (50%)	121 (69.2%)			0.45	0.26–0.77
APOB I/D	Multiplicative inheritance model					
I allele	113 (62.4%)	239 (68.3%)	0,69	0.41	1.22	(0.8–1.85)
D allele	43 (27.6%)	111 (31.7%)	(df = 1)		0.82	(0.54–1.24)
Dominant inheritance model						
II+ID	76 (47.4%)	154 (48.6 %)	5.48	0.02	5.00	(1.14–21.98)
DD	2 (2.7%)	21 (12%)	(df = 1)		0.20	(0.05–0.88)

Table 4

Distribution of the alleles and genotypes of the TCF7L2 and KCNJ11 gene markers in patients with and without CKD						
alleles/ genotypes	CKD+ (n=38)	CKD – (n = 144)	χ^2	p	OR	95% CI
TCF7L2	Multiplicative inheritance model					
G allele	16 (43.4%)	72 (50%)	1.04 (df =1)	0.31	0.77	0.46-1.28
T allele	22 (56.6%)	72 (50%)			1.30	0.78-2.17
Recessive inheritance model						
GG+GT	31 (81.6%)	134 (93.1%)	4.68 (df=1)	0.03	0.33	0.12-0.94
TT	7 (18.4%)	10 (6.9%)			3.03	1.07-8.58
KCNJ11	Multiplicative inheritance model					
A allele	23 (59.2%)	65 (45.5%)	4.54 (df = 1)	0.03	1.74	(1.04-2.91)
G allele	15 (40.8%)	79 (54.5%)			0.82	(0.34-0.96)
Recessive inheritance model						
AA	13 (34.2%)	27 (18.8%)	4.19 (df =1)	0.04	2.25	(1.02-4.97)
AG+GG	25 (65.8%)	117 (81.3%)			0.44	(0.20-0.98)

allele was associated with a predisposition to the development of CKD (OR = 1.74, 95% CI 1.04–2.91), while the *G* allele was protective (OR = 0.57, 95% CI 0.34–0.96). An analysis of the recessive inheritance model with a significance level of p= 0.04 and χ^2 = 4.19 revealed that the *A/A* genotype increased the risk of developing CKD (OR = 2.25, 95% CI 1.02– 4.97), while the presence of either the *A/G* or *G/G* genotype protected against CKD (OR = 0.44, 95% CI 0.2–0.98). These data are shown in Table 4.

No statistically significant associations of the markers of the other studied genes, i.e., the angiotensin-converting enzyme (*ACE*), apolipoprotein *E (APOE*), peroxisome proliferator activator receptor *(PPARG2*) and type 8 zinc transporter protein (*SLC30A8)* genes, with the development of CKD in T2DM patients were found in our study.

We also evaluated the incidences of CKD in patients with different combination of risk genotypes of the studied genes. These data are shown in Fig. 2.

The group 1 (n = 253) risk genotypes were as follows*:* *NOS – 4a/4a* and *APOB – I/I* or *I/D, APOE – e3/e3* and *ACE - D/D* (the latter two genes are reported according to the data in the literature data because they exhibited no reliable associations in our study). Thus, CKD developed only in 4 patients with no risk genotypes (OR = 0.23). The CKD frequencies were 25% in patients with one risk genotype (OR = 0.77), 19.4% in patients with two risk genotypes (OR = 0.47) and 35.4% in patients with three risk genotypes (OR = 1.62). The patients with the complete set of four risk genotypes exhibited the maximal CKD risk of 44% (OR = 2.13). The distribution of patients in group 2 (n = 182) exhibited a similar trend. The incidences of CKD were 17% (OR = 0.35) in the absence of risk genotypes at the *TCF7L2* and *KCNJ11* genes, 31% (OR = 0.24) in the patients with a single risk genotype and 13-fold higher (i.e., 67%, OR = 13.81) in the presence of two risk genotypes.

Discussion

The previous publications that have focused on genetic markers of renal disease in T2DM patients are very scarce and contradictory but not entirely because of the diversity of the ethnic groups or inter-population differences in the studied populations. First, the definition of renal disease in T2DM itself is associated with significant difficulties. The identification of classical DN is extremely challenging due to the wide variation in target organ damage in T2DM patients and the

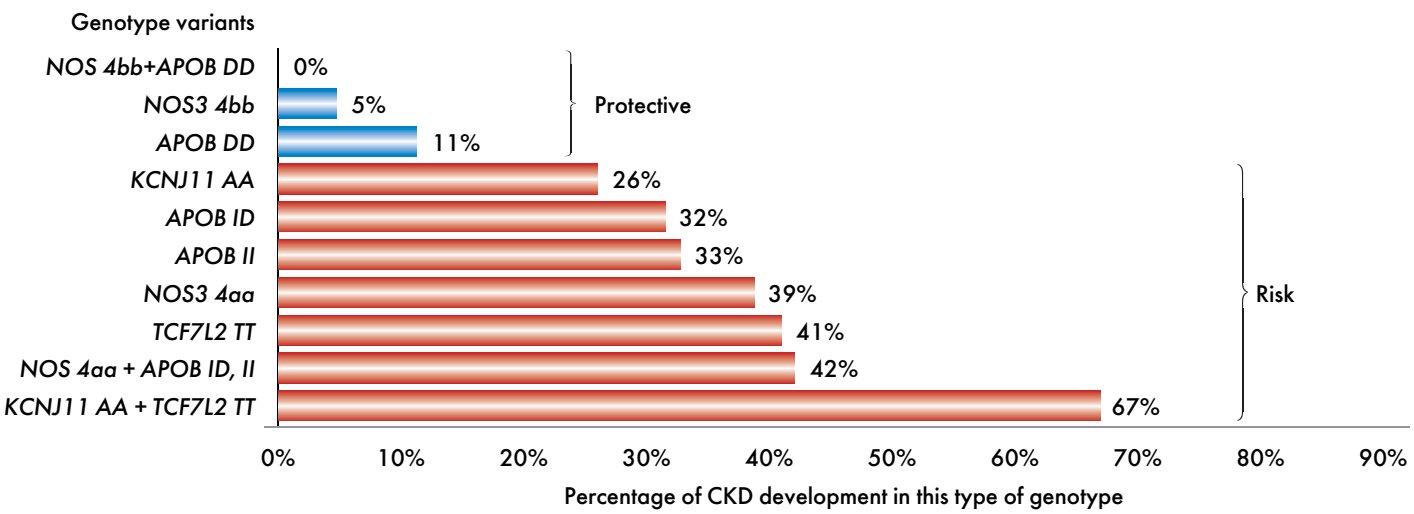

Fig. 2 The incidences of CKD in patients with risk genotypes and "protective" genotypes of the NOS3, APOB, TCF7L2 and KCNJ11 genes.

high frequencies of concomitant cardiovascular disease, hypertension, urinary tract infection and the extensive use of agents that block the renin-angiotensin system (RAS), which significantly affects the assessment of protein excretion. Thus, we suggest that the use of the CKD classification as the main criterion for experimental group formation is the best choice.

What underlies the effects of gene polymorphisms on the development of a particular disease and the consequent risk assessment and investigation? Polymorphic gene markers are variable DNA regions. The presence of a specific variant of such a region changes the gene expression product (i.e., an enzyme or transporter protein), which in turn directly or, more typically, indirectly alters some phenotypic characteristics. However, the presence of a risk allele or genotype per se does not lead to the development of the disease. Only a certain combination of alleles and genotypes enables the genetic predisposition to a pathologic phenotype under the influence of abnormal environmental factors. Because the contribution of a single gene can be negligible, the complex polygenic approach is the most promising approach to the study of genetic factors. As a rule, such studies involve polymorphic markers of genes that encode modulators of the studied pathology that are known based on the pathogenesis of the disease.

Markers of genes encoding key mediators of renal injury, including endothelial factors (e.g., the *NOS3* gene), lipid metabolism factors (e.g., the *APOB* gene) and insulin secretion factors (e.g., the *KCNJ11* and *TCF7L2* genes) exhibited significant associations with the development of CKD in our study.

The key role in the pathogenesis of renal disease in DM is traditionally assigned to endothelial dysfunction that leads to hyperactivation of RAS and impaired production of vasoprotective factors. The NO-synthase enzyme controls the synthesis of nitrogen oxide (NO), which is the main vasoprotective factor of the endothelium. The effect of the *4a* variant is associated with disorders of *NOS3* gene expression that reduce the protection afforded by NO. Our results are consistent with the literature. We have previously found an association between the *ecNOS4a/4b* marker of the *NOS* gene and the development of CKD [6] that evolves to end-stage renal disease requiring renal replacement therapy (OR = 1.74) and DN in T2DM patients (OR = 2.03) [7]. Associations with various cardiovascular pathologies, including atherosclerosis, coronary heart disease, myocardial infarction (MI) and hypertension, have also been reported for this polymorphism in T2DM patients. [8]

The *ACE* gene and its associated insertion/deletion (I/D) polymorphism is the most extensively studied gene that encoding an endothelial vasoactive factor. This polymorphism is associated with angiotensin-converting enzyme level and thus the regulation of the production of angiotensin II, which is a key factor in the development and progression of glomerulosclerosis. Currently, large amounts of data regarding the association between *I/D* polymorphisms of the *ACE* gene and vascular complications of DM, including DN, coronary artery disease and myocardial infarction, are available. According to a meta-analysis that included studies over the 10-year period and 14,727 DM patients, the *DD* genotype of the *ACE* gene is an independent risk factor for the development of DM in both types 1 and 2 DM [9]. No statistically significant differences between the groups of CKD patients were revealed in our study, which contrasts with the results of our previous studies in which *I/D* polymorphisms of the *ACE* gene were found to be strongly associated with the development of DN in T1DM patients [10]. This discrepancy might be due either to an insufficient population size for the detection of an association in T2DM patients or to the fundamentally different approach to the formation of experimental groups based on the assessment of protein excretions (in the case of T1DM) and filtration rates (in the case of T2DM). GFR and albuminuria, which are the main clinical markers of renal disease, can have different genetic determinants due to the different mechanisms of regulation.

The *APOB* gene encodes apolipoprotein B, which is structural apoprotein of the major atherogenic lipid fractions, including chylomicrons, very low density lipoproteins (VLDLs) and low density lipoproteins (LDLs), the latter of which are ligands of LDL receptors that mediate the delivery of cholesterol into cells. The role of dyslipidaemia as a predictor of renal disease progression is being extensively discussed in the literature [11]. The central role of apolipoprotein B (apoB) in the transport of lipids suggests that the carriage of certain allelic variants of the *APOB* gene might be cause of the individual differences in lipid levels. We have studied *I/D* polymorphisms of the signal peptide that is associated with the deletion of the three codons Leu-Ala-Leu at the 14-16 positions. Structural changes in this signal peptide might affect the possibility of apoB transport through membranes and thereby improve the atherogenic properties of blood. The processes of glomerulo- and atherosclerosis share common development mechanisms. In our study, the *D* allele that demonstrated significant association with CKD was found to be associated with the development of atherosclerosis [12].

The *APOE* gene encodes apolipoprotein E, which also plays an important role in lipid metabolism. The main function of apolipoprotein E is cholesterol transport from its sites of synthesis or absorption to the tissues. Apolipoprotein E exists in three isoforms, E2, E3 and E4, that are encoded by a single gene *(APOE)*. The association of the polymorphic marker ε2/ε3/ε4 of the *APOE* gene with the development of the DN in T1DM patients was revealed in our previous study [13]. The association of this marker with the development of DN in T2DM patients has been reported in foreign studies [14]. No significant association with the development of CKD in T2DM patients was found in the present study. This marker is the most complicated marker in terms of technical implementation due to the presence of three isoforms, which necessitates a significant increase in the size of the experimental population to detect any statistically significant differences.

However, the markers of the genes that have not traditionally been associated with the pathogenesis of CKD, i.e., *KCNJ11* and *TCF7L2,* are the most interesting of the present results in our view.

The Kir6.2 protein is the *KCNJ11* gene product and is one of two subunits of ATP-sensitive potassium channels that

regulate insulin secretion through the potential difference across the cell membrane. The *rs5219* polymorphic marker of the *KCNJ11* gene is associated with various phenotypes of carbohydrate metabolism disorders that range from hyperinsulinaemia and neonatal diabetes to reduced insulin secretion in T2DM. The effects of this polymorphism on the risks of developing cardiovascular complications, including CKD, are actively being discussed in connection with the fact that this type of potassium channel has been found not only in β-cells but also in the vascular wall [15]. Mutations in the *KCNJ11* gene cause structural changes to the Kir6.2 protein that which might determine individual differences in the degree of susceptibility to the pathological effects of hyperglycaemia.

The *TCF7L2* gene encodes transcription factor 7, which is similar to the factor 2, which in turn is a signalling information transfer protein in the cell. *TCF7L2* might affect the proliferation and differentiation of β-cells and importantly is involved in proglucagon gene transcription via increasing the production of glucagon-like peptide-1 (GLP-1). *TCF7L2* was traditionally associated with the development of obesity and inflammation markers. Two single-nucleotide polymorphisms (*rs12255372* and *rs7903146)* of the *TCF7L2* gene were later found to be strongly associated with the development of T2DM [16]. Hyperglycaemia and inflammation are key factors in the pathogenesis of vascular complications and kidney damage in DM. Additionally, abnormal secretion of GLP-1 is now considered not only to be a DM development factor but also the most promising predictor and therapeutic substrate of renal disease in diabetic patients [17]. The association of the *TCF7L2* gene with CKD has been shown in the Japanese population [18]. We would also like to mention a study that examined the relationships between 18 genetic T2DM markers and the development of nephropathy. This study demonstrated associations between markers of the *KCNJ11* and *TCF7L2* genes and low GFR levels but not with albuminuria [19], which supports our hypothesis of different genetic determinants of CKD clinical markers.

Concomitant genetic predispositions to the development of DM have been demonstrated for several classical candidate genes of renal disease *(*e.g., *ACE* and *NOS3)* that might be indicative of common pathogenetic mechanisms of DM and its complications. Therefore, we included the *PPARG2* and *SLC30A8* genes in our search for genetic predispositions to CKD.

The *PPARG2* gene encodes a nuclear receptor that is involved in the regulation of the synthesis of fatty acids, TNF-α, resistin, adiponectin and adipogenesis factors. The PPARγ complex with the retinoid X receptor (RXR) binds to the PPAR-binding structural elements in the promoter regions of target genes and regulates their expression. Mutations in the *PPARG* gene cause PPARγ ligand-resistant syndrome, which manifests as a complex of clinical symptoms that includes insulin resistance, dyslipidaemia, hypertension, increased body weight and inadequate glucose homeostasis. The associations of *PPARG2* gene polymorphism with T2DM have been shown [20]. No association of the gene with the risk of CKD was observed in our study.

The type 8 zinc transporter protein (ZnT-8) is a product of the *SLC30A8* gene that regulates the concentration of zinc ions in β-cells and is necessary for insulin secretion. Zinc plays an important role in the regulation of the ripening, storage and secretion of insulin by β-cells. The involvement of the *SLC30A8* gene in the development of T2DM have been shown in several large-scale studies [20]. Unfortunately, we found no association of this gene with the risk of CKD.

When calculating the odds ratios for CKD, we found that the risk of developing CKD depends on the number of risk genotypes. The relative risk of CKD was minimal in the absence of risk genotypes (i.e., the presence of only protective variants; ORs = 0.23 and 0.35 in the 1st and 2nd groups, respectively) and progressively increased with the number of risk genotypes to ORs of 2.13 and 13.8. Of course, these data are insufficient to grade CKD risk, and further research with larger populations is required. Nevertheless, our results again confirm the polygenic nature of the development of CKD.

Conclusions

This study showed that the development of CKD in T2DM patients is genetically determined. We found that there were significant associations between CKD risk and the genes that encode the endothelial factors *(NOS3),* lipid metabolism factors *(APOB)* and insulin secretion factors (*KCNJ11* and *TCF7L2)* whose expression products are involved in the major pathogenetic mechanisms of renal disease in DM. The combinations of risk genotypes were critically important for this complex analysis of the studied markers. The risk of CKD was very low when only protective genotypes were present and progressively increased with the accumulation of risk genotypes; these findings suggest that this panel of polymorphic markers can be used as genetic diagnostic kit for the prediction of CKD and the formation of risk groups for this pathology at the preclinical stage.

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