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# Genetic variants in *SLC9A9* gene coding for sodium/hydrogen exchanger 9 are not associated with diabetic kidney disease

Brak związku wariantów genetycznych w genie *SLC9A9* kodującym antyporter sodowo-protonowy 9 z cukrzycową chorobą nerek

## ABSTRACT

**Background.** Several independent studies found a linkage between diabetic kidney disease and chromosome 3q22. Following studies were not able to find a genetic polymorphism in the chromosome 3q22 region which was associated with diabetic kidney disease and which could explain the observed linkage. The *SCL9A9* gene coding for the sodium/hydrogen exchanger 9 is located in the critical region on chromosome 3q22. Genetic variants of the *SCL9A9* gene might be involved in the abnormal kinetics of erythrocyte sodium-lithium countertransport observed in patients with diabetic kidney disease. The aim of the study was to check the association between genetic polymorphisms of the *SCL9A9* gene and diabetic kidney disease. **Material and methods.** We collected 61 patients with diabetic kidney disease and 63 patients with normoalbuminuria after at least 15 years of known diabetes duration. Peripheral blood was drawn and DNA was extracted from leukocytes. Fragments of the *SCL9A9* gene were amplified by PCR and digested by specific restriction enzymes. Altogether 3 poly-

morphisms were genotyped: rs17594058 in intron 1, rs7641634 in intron 2 and rs6763202 in exon 8. The genotype frequency was compared between patients with and without diabetic kidney disease.

**Results.** There was no difference in the genotype frequency for analyzed polymorphisms in the *SCL9A9* gene between patients with and without diabetic kidney disease.

**Conclusions.** The genetic variants of the *SCL9A9* gene localized in the critical region on chromosome 3q22 are not associated with diabetic kidney disease. (Diabet. Klin. 2014; 3, 1: 17–21)

**Key words:** diabetic kidney disease, genetic predisposition, chromosome 3q22

## STRESZCZENIE

**Wstęp.** W kilku niezależnych badaniach stwierdzono sprzężenie pomiędzy fragmentem chromosomu 3q22 a cukrzycową chorobą nerek. Kolejne badania nie były w stanie znaleźć polimorfizmu genetycznego zlokalizowanego na chromosomie 3q22, który wykazywałby związek z cukrzycową chorobą nerek i tłumaczyłby wcześniej stwierdzone sprzężenie. Gen *SCL9A9* kodujący antyporter sodowo-protonowy izoformę 9 jest zlokalizowany w krytycznym regionie na chromosomie 3q22. Warianty genetyczne w genie *SCL9A9* mogą być odpowiedzialne za odmienną kinetykę transportu sodowo-litowego obserwowanego w erytrocytach u chorych z cukrzycową chorobą nerek. **Celem badania** była analiza związku pomiędzy polimorfizmami genu *SCL9A9* a cukrzycową chorobą nerek.

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**Materiał i metody.** Do badania włączono 61 chorych z cukrzycową chorobą nerek oraz 63 chorych z prawidłowym wydalaniem albumin w moczu po co najmniej 15 latach trwania cukrzycy. Od każdego chorego pobrano krew obwodową i z leukocytów wyizolowano DNA. Fragmenty genu *SCL9A9* namnażano w reakcji PCR i trawiono specyficznymi enzymami restrykcyjnymi. W sumie przeprowadzono genotypowanie 3 polimorfizmów: rs17594058 w intronie 1, rs7641634 w intronie 2 oraz rs6763202 w egzonie 8. Porównano częstość występowania genotypów pomiędzy chorymi z i bez cukrzycowej choroby nerek.

**Wyniki.** Nie stwierdzono różnic w częstotliwości genotypów badanych polimorfizmów genu *SCL9A9* pomiędzy chorymi z i bez cukrzycowej choroby nerek.

**Wnioski.** Warianty genetyczne w genie *SCL9A9* zlokalizowanym w krytycznym regionie na chromosomie 3q22 nie wykazują związku z cukrzycową chorobą nerek. (Diabet. Klin. 2014; 3, 1: 17–21)

**Słowa kluczowe:** cukrzycowa choroba nerek, predyspozycja genetyczna, chromosom 3q22

## Introduction

Epidemiological studies indicated a genetic predisposition to diabetic kidney disease [1, 2]. Two independent studies found a linkage between diabetic kidney disease and region on chromosome 3q22 [3, 4]. Both of those studies used the same family model for linkage analysis based on sib-pairs concordant for type 1 diabetes, but discordant for diabetic kidney disease.

Several studies found an increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and diabetic kidney disease. The etiology of increased sodium-lithium countertransport activity in red cells in patients with diabetic kidney disease was not found [5, 6]. Increased activity of one of the isoforms of sodium/hydrogen exchanger was suspected to be associated with that phenotype. Two isoforms were analyzed, but no association was found between increased sodium-lithium countertransport activity in red cells and sodium/hydrogen exchanger 1 and 3. The *SLC9A9* gene coding sodium/hydrogen exchanger 9 is localized on chromosome 3q22 in the critical region linked with diabetic kidney disease. The association between genetic variants of the sodium/hydrogen exchanger 9 gene and diabetic kidney disease has not been analyzed. The role of the sodium/hydrogen exchanger 9 in the etiology of increased sodium-lithium countertransport activity in red cells in patients with diabetic kidney disease is also not known.

There is a strong difference in predisposition to diabetic kidney disease among different ethnic groups. The lower risk for diabetic kidney disease is observed in Caucasian and the highest in African-American and Native Indian. The difference in incidence of diabetic kidney disease may be explained both by genetic drift or adaptive evolution. In a recent report, 101 regions of the human genome were identified with very strong evidence of a recent selective sweep in adaptive evolution [7]. One of them is the 5' end of the *SLC9A9* gene on chromosome 3q22 with a strong selective sweep in European-American and Chinese.

The aim of the study was to analyze the association between diabetic kidney disease and genetic variants in the *SLC9A9* gene.

## Patients and methods

The study included 61 diabetic patients with diabetic kidney disease (albumin/creatinine ratio,  $ACR \geq 30$  mg/g) and 63 diabetic patients with normoalbuminuria ( $ACR < 30$  mg/g) after at least 15 years of known diabetes duration. Patients without diabetic retinopathy were excluded from the diabetes kidney disease group. 31 patients in the diabetic kidney disease group had moderately increased albuminuria ( $ACR 30-300$  mg/g) and 30 had severely increase albuminuria ( $ACR > 300$  mg/g). We excluded also from the diabetic kidney disease group patients with increased numbers of erythrocytes and leukocytes in urine. For each patient, 2.7 ml of blood were collected from peripheral vein. Erythrocytes were lysed with lysis buffer and DNA was extracted from leukocytes using the Genomic Midi AX 20 set (A&A Biotechnology, Gdynia, Poland). Three polymorphisms localized in the *SLC9A9* gene were genotyped: rs17594058 in intron 1, rs 7641634 in intron 2 and rs6763202 in exon 8. Following primers and restriction enzymes were used for genotyping:

- rs17594058 F 5'-ggg act taa gag gga cat Ctt a-3',  
rs17594058 R1 5'-cag cag agt gta cct gca ct-3'  
(MolBiol, Poznan, Poland), *HpyF31* (Fermentas, Lithuania);
- rs7641634 F 5'-cta ggt ctg ttt agt ggt cGc att-3',  
rs7641634 R1 5'-aag aca gga cag agt agc ag-3'  
(MolBiol, Poznan, Poland), *Mva1269I* (Fermentas, Lithuania);
- rs6763202 R 5'-gtt tcc agc atc ggg aac Gc-3';  
rs6763202 F1 5'-gag cta tca tca cca gat cag-3'  
(MolBiol, Poznan, Poland), *Bsh1236I* (Fermentas, Lithuania).

Underlined nucleotide indicates a DNA mismatch introduced into the primer to provide a specific restriction site. The DNA fragment containing analyzed polymorphism was amplified by PCR. Amplified fragments

**Table 1. Clinical characteristics of patients with diabetic kidney disease (DKD) and without diabetic kidney disease (No DKD). Data are presented as median (minimum and maximum)**

	DKD	No DKD	p value
n	61	63	
Gender (female/male)	31/30	32/31	NS
Diabetes (type 1/type 2)	14/47	25/38	NS
Age (years)	62 (24–100)	56 (21–90)	NS
Diabetes duration (years)	15 (1–42)	20 (15–40)	< 0,01
ACR [mg/g]	21 (32–6799)	6 (0–29)	< 0,001
Serum creatinine [ $\mu\text{mol/L}$ ]	122 (67–555)	94 (68–197)	< 0,001
HbA <sub>1c</sub> (%)	8.0 (5.4–13.5)	7.5 (5.4–12.2)	NS
BMI [ $\text{kg/m}^2$ ]	29.9 (21.7–62.7)	26.4 (19.0–41.0)	NS
Insulin treatment (yes/no)	55/6	59/4	NS
Triglycerides [ $\mu\text{mol/L}$ ]	1.7 (0.6–4.8)	1.3 (0.4–4.7)	NS
Total cholesterol [ $\mu\text{mol/L}$ ]	4.89 (2.1–9.1)	4.6 (2.4–7.7)	NS
Smoking (yes/no)	18/43	11/52	NS
ACEI or AT1B (yes/no)	40/21	30/33	NS
Retinopathy (yes/no)	61/0	26/37	< 0.001

**Table 2. Genotype frequency in patients with diabetic kidney disease (DKD) and without diabetic kidney disease (No DKD)**

rs17594058 (intron 1)	GG	AG	AA	p value
No DKD (n = 63)	22 (35%)	33 (52%)	8 (13%)	
DKD (n = 61)	25 (41%)	29 (48%)	7 (11%)	NS
rs7641634 (intron 2)	CC	CT	TT	p value
No DKD (n = 63)	40 (63%)	20 (32%)	3 (5%)	
DKD (n = 61)	41 (67%)	16 (26%)	4 (7%)	NS
rs6763202 (exon 8)	TT	CT	CC	p value
No DKD (n = 63)	34 (54%)	27 (43%)	2 (3%)	
DKD (n = 61)	24 (39%)	33 (54%)	4 (7%)	NS

were digested with restriction enzyme recognizing only one of two alleles. PCR products were separated by 3% agarose gel electrophoresis.

Genotype frequency was compared between patients with and without diabetic kidney disease.

Statistical analysis was performed using software R version 2.15.2 (The R Foundation for Statistical Computing). The R package 'genetics' was used to calculate linkage disequilibrium between genotyped polymorphisms (<http://rgenetics.org>).

The Ethics Committee of the Medical University of Lodz approved the study protocol.

## Results

The clinical characteristics of patients analyzed in the study is presented in Table 1. Patients with diabetic kidney disease had by design higher urine albumin excretion than those without diabetic kidney. They had

shorter known duration of diabetes and higher serum creatinine concentration. Patients with increase urine albumin excretion had to have retinopathy to be included into the diabetic kidney disease group.

The genotype frequency is presented in Table 2. There were no significant differences in the genotype frequency between patients with and without diabetic kidney disease for the rs17594058 polymorphism in the intron 1, the rs7641634 polymorphism in the intron 2 and the rs6763202 polymorphism in the exon 8 of the *SLC9A9* gene.

The linkage disequilibrium between rs17594058, rs7641634 and rs6763202 polymorphisms in the *SLC9A9* gene is presented in Table 3. There were a strong linkage disequilibrium between the rs17594058 polymorphism in the intron 1 and the rs7641634 polymorphism in the intron 2 ( $D' = 0,99$ ). The linkage disequilibrium between the rs7641634 polymorphism

**Table 3. Linkage disequilibrium between genotyped polymorphisms in the *SCL9A9* gene**

	rs17594058 (intron 1)	rs7641634 (intron 2)
rs7641634 (intron 2)	0.99 (p < 0.001)	
rs6763202 (exon 8)	0.04 (NS)	0.52 (p < 0.01)

in the intron 2 and the rs6763202 polymorphism in the exon 8 was weak ( $D' = 0,52$ ). No linkage disequilibrium was observed between the rs17594058 polymorphism in the intron 1 and the rs6763202 polymorphism in the exon 8 ( $D' = 0,04$ ).

## Discussion

We did not find an association between diabetic kidney disease and polymorphisms in the *SLC9A9* gene. The *SLC9A9* was a good candidate gene in the critical region on the chromosome 3q22 linked to diabetic kidney disease. The question stays open which genetic variant in the critical region on chromosome 3q22 is associated with diabetic kidney disease.

Chromosome 3q22 was linked with diabetic kidney disease [3, 4]. Several studies tried to find a genetic marker for diabetic kidney disease in the 3q22 region [8–11]. More than 3000 single nucleotide polymorphism localized in the critical 3q22 region were analyzed in 1822 type 1 diabetes patients with diabetic kidney disease and 1874 type 1 diabetes patients without diabetic kidney disease [12]. One of the analyzed genetic variants the rs1866813 polymorphism was associated with diabetic kidney disease. The observed association between the rs1866813 polymorphism and diabetic kidney disease seems to be too weak to explain previously observed linkage [3, 4]. The association between the rs1866813 polymorphism and diabetic kidney disease has not been confirmed in Polish population [13]. Confirmatory reports from other populations are also missing. Only one whole-genome association study was published for diabetic kidney disease [14]. In that study no polymorphism in the critical region of the 3q22 polymorphism was found to be associated with diabetic kidney disease [14].

The role of the sodium/hydrogen exchanger 9 has not been well explained. In one study chromosomal aberration affecting the *SLC9A9* gene and in another one genetic variants in the *SLC9A9* gene were associated with an attention-deficit/hyperactivity disorder symptoms [15, 16]. Sodium/hydrogen exchanger 9 shows high homology with other sodium/hydrogen exchanger including the sodium/hydrogen exchanger 1 and 3 previously analyzed in diabetic kidney disease.

Increased erythrocyte sodium lithium countertransport observed in patients with diabetic kidney disease could not be inhibited by amiloride [17]. The role of the sodium/hydrogen exchanger 1 and 3 in the etiology of increased sodium lithium countertransport is less probable, because they can be inhibited by amiloride [17]. The effect of amiloride on inhibition of the sodium/hydrogen exchanger 9 and the presents of the sodium/hydrogen exchanger 9 in human erythrocytes has not been examined yet. The question stays open what is responsible for the phenotype of increased sodium-lithium countertransport activity in red cells in diabetic kidney disease.

One of the reasons why we analyzed the *SLC9A9* gene is a strong selective sweep in European-American and Chinese at the 5' end of the *SLC9A9* gene on chromosome 3q22. This selective sweep might explain why there are significant difference in predisposition to diabetic kidney disease among different ethnic groups. Negative results of the study did not confirm the primary hypothesis that genetic variants in the *SLC9A9* gene might explain association between predisposition to diabetic kidney disease and ethnicity.

In conclusion, we were not able to prove that the *SLC9A9* gene coding the sodium/hydrogen exchanger 9 is associated with genetic predisposition to diabetic kidney disease.

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Conflict of interest statement — none declared.

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