PRACA ORYGINALNA

Lack of association between single nucleotide polymorphisms of *CPA4, LEP* and *AKR1B1* genes located at the long arm of chromosome 7 (7q31-q35) and chronic kidney disease occurrence and progression

Brak związku między polimorfizmami pojedynczego nukleotydu genów *CPA4, LEP* oraz *AKR1B1* zlokalizowanych na długim ramieniu chromosomu 7 (7q31-q35) a występowaniem i progresją przewlekłej choroby nerek

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ABSTRACT

BACKGROUND

The aim of the study was to investigate the influence of single nucleotide polymorphisms (SNPs) of carboxypeptidase A4, *CPA4*, leptin, *LEP* and aldo-keto reductase family 1, *AKR1B1* genes located at the long arm of chromosome 7 (7q31-q35) on development and progression of chronic kidney disease (CKD).

MATERIAL AND METHODS

There was an association study by PCR-RFLP method of following SNPs in parent-offspring trios performed: G934T of *CPA4* gene, A19G of *LEP* gene and C-106T of *AKR1B* gene. 471 subjects, 157 patients with CKD and 314 their biological parents were examined. The patients were divided into 3 groups: diabetic nephropathy due to type 1 diabetes (n = 34), chronic primary glomerulonephritis (n = 70) and chronic interstitial nephritis (n = 53). The mode of alleles transmission was determined using the transmission disequilibrium test (TDT).

RESULTS

There was no association of studied SNPs and CKD occurrence or progression rate of renal function loss. Transmission of alleles of investigated SNPs did not differ significantly: G934T of CPA4 gene: P = 0.61 in whole group of CKD patients, p = 0.66 in GN group, p = 0.70 – IN group and

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Ann. Acad. Med. Siles. 2012, 66, 2, 27–33

ISSN 0208-5607

p = 0.61 in DN one; A19G of LEP gene: p = 0.58, 0.71, 0.78 and 0.49, respectively; C-106T of ALDR1 gene: p = 0.31, 0.47, 0.12 and 0.38, respectively. No impact of examined polymorphisms on the rate of progression of renal function loss was observed.

CONCLUSIONS
The results, obtained in the study, suggest that the investigated SNPs: G934T of *CPA4* gene, A19G of *LEP* gene and C-106T of *AKR1B* gene may not play a major role in the development and progression of chronic nephropathies.

KEY WORDS
chronic kidney disease, gene polymorphism, TDT, SNP

STRESZCZENIE

WSTĘP
Celem badań było zbadanie wpływu polimorfizmów pojedynczego nukleotydu (SNPs) genów karboksypepsydazy A4, *CPA4*, leptyny, *LEP* i reduktazy aldozy, *AKR1B1*, znajdujących się na długim ramieniu chromosomu 7 (7q31-q35) na rozwój i progresję przewlekłej choroby nerek (PChN).

MATERIAŁ I METODY
Wykorzystując metodę PCR-RFLP przebadano następujące polimorfizmy: G934T *CPA4* genu, A19G *LEP* i C-106T genu *AKR1B*. Badaniami objęto 471 osoby: 157 z PChN i 314 ich biologicznych rodziców. Pacjentów podzielono na 3 grupy: z nefropatią cukrzycową w przebiegu cukrzycy typu 1 (DN, n = 34), z przewlekłym pierwotnym kłębuszkowym zapaleniem nerek (GN, n = 70) oraz z przewlekłym śródmiąższowym zapaleniem nerek (IN, n = 53). Tryb przekazywania alleli został oceniony testem nierównowagi przekazywania (Transmission-Disequilibrium Test, TDT).

WYNIKI
Częstość przekazywania alleli analizowanych SNPs nie odbiegała znacząco od oczekiwanej: G934T *CPA4*: p = 0,61 w całej grupie badanej, p = 0,66 w grupie GN, p = 0,70 – w grupie IN oraz p = 0,61 w grupie DN; A19G *LEP*: p = 0,58; 0,71; 0,78 i 0,49, odpowiednio; C-106T genu *ALDR1*: p = 0,31; 0,47; 0,12 i 0,38, odpowiednio. Nie zaobserwowano żadnego wpływu badanych polimorfizmów na szybkość utraty funkcji nerek.

WNIOSKI
Uzyskane w badaniu wyniki wskazują, że badane SNPs: G934T genu *CPA4*, A19G *LEP* i C-106T genu *AKR1B* nie odgrywają istotnej roli w rozwoju i progresji przewlekłych nefropatii.

SŁOWA KLUCZOWE
przewlekła choroba nerek, TDT, polimorfizm genowy, polimorfizm pojedynczego nukleotydu (SNP)

BACKGROUND

Chronic kidney disease (CKD) prevalence is high and the disease is associated with significant morbidity and is the direct risk factor for cardiovascular complications development. The environmental risk factors of CKD occurrence and progression that have been recognized up to date are not sufficient enough for identification of groups of people at higher risk of the disease development as well as to develop new and efficient treatment methods. In a view of foregoing there are a lot of expectations put on the genetic factors, along with environmental ones, related to the CKD occurrence and progression [1,2,3]. Genetic factors frequently manifest only in the presence of special situations such as presence of other diseases for example diabetes mellitus or hypertension. However, not everyone who suffers from these conditions develops renal disease or progresses to ESRD, that is why it is likely that genetic factors determine the time of onset and the rate of progression of CKD.

Due to the hypothesis that common genetic variants predispose to common diseases there is a rise in interesting in single nucleotide polymorphism (SNP) [4]. One of the approaches for association studies of complex traits is called the transmission/disequilibrium test (TDT). The test examines the transmission of a particular molecular variant (allele) from heterozygous parents to affected offspring, and the observed transmission is compared with the transmission expected for no association (that is, random transmission of 50:50% for two-allele systems) [5,6].

The aim of the study was to evaluate the role of polymorphisms of the genes encoding for protein that are connected with a physiologic kidney function or development and/or progression of CKD located on long arm of chromosome 7, namely G934T of *CPA4* gene, A19G of *LEP* gene and C-106T of *AKR1B* gene.

MATERIAL AND METHODS

SELECTION OF FAMILY TRIOS

Subjects for the study were requited from 17 Nephrology or Dialysis centres in Poland. From 1657 Caucasian patients with a history of CKD in stage 4. at least (estimated glomerular filtration rate, eGFR < 30 ml/min/1.73m2) or undergoing chronic haemodialysis or peritoneal dialysis there were 157 patients selected who had both parents alive: 70 patients with primary glomerulonephritis (GN group), 53 patients with interstitial nephritis (IN group) and 34 patients with diabetic nephropathy in type 1 diabetes mellitus (DN group). There were family trios with patients with end stage renal disease (ESRD) due to heritable kidney diseases and unknown origin excluded. A detailed history of kidney disease was collected from all patients and all parents providing basic epidemiological data.

All patients and parents gave written informed consent and the study was carried out in accordance with the Declaration of Helsinki, and the protocol approved by the University Ethics Committee.

DIAGNOSIS OF UNDERLYING ETIOLOGY OF CKD

Diagnosis of diabetic nephropathy was made after examination of urinary albumin (obtaining in two out of three measurements an outcome of 30–299 mg albumin in 24 hour urine collection or ≥ 300 mg of protein in 24-hour urine collection) and analysis of clinical history (lack of other clinical and laboratory signs of kidney or urinary tract disease). Urinary albumin was measured using a commercially available kit. The urine collection procedure was performed from 8:00 p.m. to 8:00 a.m. during three consecutive days.

Diagnosis of chronic glomerulonephritis was made on the basis of clinical history and existence of persistent proteinuria and/or hematuria or cylinduria as well as coexisting decreased of eGFR value. Additionally, in the group of 13 patients diagnosis was confirmed by kidney biopsy.

Diagnosis of chronic interstitial nephritis was made on the basis of clinical history confirming existence of recurrent urinary tract infections, pathological image in USG examination and/or urography examination and pathological urine sediment.

DNA ANALYSIS

From all patients and parents, genomic DNA was isolated from peripheral blood leukocytes. Genotyping was performed in a blinded fashion.

1. Genotyping of G934T polymorphism of *CPA4* gene

Genomic DNA was extracted from frozen whole blood samples containing EDTA as an

anticoagulant by use of the MasterPure TM DNA purification kit (Epicentre Technologies) and resuspended in TE. Primers (Epicentre Technologies) used for amplification were as follows: forward 5' CGA CAA CCC TTG CTC CGA AGT G-3'; and reverse: 5'-TAG CTG TGC AGG TCG ATG AGG C'. PCR amplification was performed in a total volume of 25 µl which contained 100 ng of genomic DNA. Reaction mixture contained: 10 mmol/l Tris HCL, PH 8.8, 50 mmol/l KCL, 1.5 mmol/l MgCl2, 0.1% Triton X-100, 2.5 mmol/l of each deoxynucleotide triphosphate, 10 pmol of each primer, 1.5 mmol L–1 magnesium chloride and 0.6 U of termostable Taq DNA polymerase (DyNAzyme TM II, Finnzymes). PCR amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 60°C for 1min, and extension at 72°C for 1min., and a final extension at 72°C for 7 min. PCR products were digested with EcoO109I enzyme for the detection of G934T polymorphism of *CPA4* gene. All samples were analysed on 2% agarose gel electrophoresis and visualized by ethidium bromide staining in UV light (Vilber Loumat transilluminator UV).

2. Genotyping of A19G polymorphism of *LEP* gene

Genomic DNA was extracted from frozen whole blood samples containing EDTA as an anticoagulant by use of the MasterPure TM DNA purification kit (Epicentre Technologies) and resuspended in TE. Primers (Epicentre Technologies) used for amplification were as follows: forward 5' CCC GCG AGG TGC ACA CTG-3'; and reverse: 5'-AGG AGG AAG GAG CGC GCC-3'. PCR amplification was performed in a total volume of 25 µl which contained 100 ng of genomic DNA. Reaction mixture contained: 10 mmol/l Tris HCL, PH 8.8, 50 mmol/l KCL, 1.5 mmol/l MgCl2, 0.1 % Triton X-100, 2.5 mmol/l of each deoxynucleotide triphosphate, 10 pmol of each primer, 1.5 mmol L–1 magnesium chloride and 0.6 U of termostable Taq DNA polymerase (DyNAzyme TM II, Finnzymes). PCR amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 60°C for 1min, and extension at 72°C for 1min., and a final extension at 72°C for 7 min. PCR products were digested with TaaI (Fermentas) enzyme for the detection of A19G polymorphism of *LEP* gene. All samples were analysed on 2% agarose gel electrophoresis and visualized by ethidium bromide staining in UV light (Vilber Loumat transilluminator UV).

3. Genotyping of C-106T polymorphism of *AKR1B1* gene

Genomic DNA was extracted from frozen whole blood samples containing EDTA as an anticoagulant by use of the MasterPure TM DNA purification kit (Epicentre Technologies) and resuspended in TE. Primers (Epicentre Technologies) used for amplification were as follows: forward 5'- CAG ATA CAG CAG CTG AGG AAC-3'; and reverse: 5'-GCC TTC TGA TTG GTT GCA CT-3'. PCR amplification was performed in a total volume of 25 µl which contained 100 ng of genomic DNA. Reaction mixture contained: 10 mmol/l Tris HCL, PH 8.8, 50 mmol/l KCL, 1.5 mmol/l MgCl2, 0.1 % Triton X-100, 2.5 mmol/l of each deoxynucleotide triphosphate, 10 pmol of each primer, 1.5 mmol L–1 magnesium chloride and 0.6 U of termostable Taq DNA polymerase (DyNAzyme TM II, Finnzymes). PCR amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 60°C for 1min, and extension at 72°C for 1min., and a final extension at 72°C for 7 min. PCR products were digested with BseNI enzyme for the detection of C-106T polymorphism of *AKR1B1* gene. All samples were analysed on 2% agarose gel electrophoresis and visualized by ethidium bromide staining in UV light (Vilber Loumat transilluminator UV).

OTHER DETERMINANTS AND DATA PROCESSING

Anthropometric measurements (height and weight) were measured by standard methods. Serum creatinine was measured by Jaffe's method (Cobas Integra 800, Roche Diagnostics).

All patients from study group had BMI calculated as weight/height^2 (kg/m^2) and the (eGFR) per 1.73 m^2 estimated according to MDRD [7]: 186 × {serum creatinine (mg/dl)} – 1.154 × {age (years)} – 0.203 × (0.742 if woman) and according to Schwartz equation if the patient was under the age of 18 [8]: coefficient x {body length (cm)} / {serum creatinine (mg/dl)}. Where coefficient was 0.45 if < 2 years of age, 0.55 if > 2 years of age and 0.7 if ≥ 13 years of age. Stages of CKD were defined according to standards [9]. It was presupposed that in order to count

progression of CKD patients follow up cannot be shorter than 6 months.
Progression of CKD was assessed as mean reduction of eGFR per year. Reduction of eGFR $\geq$ 4 ml/min/1.73 m^2/year was assumed as severe progression of CKD and reduction of eGFR $\leq$ 4 ml/min/1.73 m^2/year was assumed as benign progression of CKD because the value 4 ml/min/1.73 m^2 was the closest to median annual eGFR reduction counted for the whole group and subgroup of patients with CKD.

STATISTICS

All statistic calculations were performed using Microsoft Office Excel 2003 and Statistica 8.0, StatSoft Inc (USA).
Shapiro-Wilk test for normality was used. Descriptive statistics for continuous parameters of normal distribution were arithmetic means ± SD (standard deviation) or geometric means (interquartile range) for continuous data that did not have normal distribution. Categorical variables were absolute value and percentage.
Accordance with Hardy-Weinberg was tested with Pearson 2 test with Yates correction.
Difference among categorical variables was assessed by Pearson $\chi 2$ test or Fisher exact test.
Difference among continuous variables was tested by ANOVA (analysis of variance) for single classification with post hoc analysis of least significant differences. To assess the equality of variances in different samples Levene test was used.
In the TDT, the observed transmission of alleles from heterozygous parents to affected offspring was compared with an expected proportion of 50% transmission for an allele not associated with the phenotype, and McNemar's test was used for the comparisons.
Patients had the retrospective history of repeated measurements (at least five) of serum creatinine from the onset of CKD during a follow time of at least year collected.
For each patient the reciprocal serum creatinine concentration was plotted versus time between measurements by means of the least-squares regression method. For each case, such a plot fitted the model of linear regression, with correlation coefficients varying from – 0.884 to -0.997 ($P < 0.05$) and the slope was used to define the rate of progression of renal function loss. Mean regression coefficients were compared between subgroups of patients carrying different genotypes in the examined loci using the parallelity test.
The two-tailed statistical significance was set at $p < 0.05$.

RESULTS

Among 157 patients enrolled in the study, 104 of them were treated with renal replacement therapy (93 patients on maintenance haemodialysis and 11 peritoneal dialysis) and 53 patients were treated conservatively. Median follow up time from the beginning of observation till the start of renal replacement therapy was 8 years (3.0–13.0 years). The median of first documented eGFR value at the diagnosis of CKD was 39,5 ml/min/1,73m^2 (14.4–70.9 ml/min/1.73m^2).
Selected clinical data of patients from study group are presented in Table 1.

Table 1. Selected clinical data of patients from study group
Tabela 1. Charakterystyka grupy badanej – wybrane dane kliniczne

Selected clinical data	Total	DN	GN	IN
n	157	34	70	53
Age (years)	27.5 ± 12.5	38.0	28.8 ± 11.2	19.0 ± 9.7
eGFR at the beginning of observation of CKD (ml/min/1.73m2)	39.5	50.8	60.6	33.6

DN – diabetic nephropathy; GN – chronic primary glomerulonephritis; IN – interstitial nephritis

Genotypic proportions of studied polymorphisms were in Hardy-Weinberg equilibrium.
There was no association of studied SNPs and CKD occurrence or progression rate of renal function loss. Transmission of alleles of investigated SNPs did not differ significantly: G934T of *CPA4* gene: P = 0.61 in whole group of CKD patients, p = 0.66 in GN group, p = 0.70 – IN group and p = 0.61 in DN one; A19G of *LEP* gene: p = 0.58, 0.71, 0.78 and 0.49, respectively; C-106T of *ALDR1* gene: p = 0.31, 0.47, 0.12 and 0.38, respectively.
No impact of examined polymorphisms on the rate of progression of renal function loss was observed.

DISCUSSION

TDT allows disclosure of the impact of genes of even a slight effect on the phenotype [10,11].

Despite this, in our study the results of TDT analysis for the SNPs have not shown statistically significance in differences of transmission of investigated alleles.

Carboxypeptidase A4 (CPA4) is a member of the metallocarboxypeptidase family. CPA4 mRNA expression is associated with hormone-regulated tissues, suggesting that it may have a role in cell growth and differentiation [12]. The human CPA4 gene is located on chromosome 7q32, which is a region in the genome that might contain genes for prostate cancer aggressiveness. Expression of CPA4 was found – among other – in kidney tissues [13,14]. This enzyme is important in the regulation of peptides like kinins and plays role in activation of inflammation processes and regulation of glomerular filtration. Effects of kinins in the kidney are mainly mediated by the bradykinin B2-receptor, and have influence on natriuresis, vasodilatation and arterial blood pressure. It was found that bradykinin B2 receptor activation reduces renal fibrosis [15,16].

We didn't observe, as in other studies, effects of genetic variation of CPA4 gene on development and progression of CKD.

Leptin, the product of the obesity (ob) gene, cytokine is produced by adipocytes. Hyperleptinemia which is observed in overweight persons is associated with functional and structural changes in the kidneys [17,18]. Proteinuria is observed in more than 90% obese individuals with a BMI above 30 kg/m^2 [19]. Glomerulomegaly and focal segmental glomerulosclerosis are the most typical structural signs of obesity-related nephropathy [18,20,21,22]. Leptin stimulates expression of TGF-beta1, has mitogenic and fibrotic action and causes accumulation of collagen I and IV in the mesangium [19,23,24,25].

Through adrenergic activation and modification of natriuresis leptin raises arterial blood pressure [26,27]. The kidney is one of the few extra-neural tissues that express the leptin receptors. They are responsible for the diuretic and natriuretic action of the hormone [24].

Despite such data, indicating the possible impact of leptin on pathophysiology of kidney diseases, effect of *LEP* polymorphisms on development of CKD so far it has not been demonstrated. The results of my tests also do not confirm such a hypothesis.

Aldose reductase (AKR1B1) is a cytosolic enzyme that, in the presence of NADPH, catalyses the rate-limiting step of the polyol pathway converting glucose into sorbitol, especially under hyperglycemic condition. The enzyme is located in the eye (cornea, retina, and lens), kidney, and the myelin sheath–tissue [29,30]. In this context, *AKR1B1* is a natural candidate gene potentially responsible for development and progression of diabetic nephropathy. C106T *AKR1B1* polymorphism was identified in both Caucasian and Asian subjects with type 1 or type 2 diabetes, and association with diabetic nephropathy has been observed [30,31,32,33].

However, there are reports which suggest that C106T AKR1B1 polymorphism doesn't play a role in diabetic nephropathy development [34,35,36].

The results of our investigations do not confirm the influence of AKR1B1 C106T polymorphism on the development and progression of CKD. However, it should be stressed that the examined group of type 1 diabetic subjects had only 34 persons.

Prevalence of "silent" CKD is high among people with diagnosed and undiagnosed diabetes, chronic glomerulonephritis and arterial hypertension. These individuals might benefit from interventions aimed at preventing development and/or progression CKD.

In this context, the detection of novel candidate-genes responsible for development or progressions CKD and identifying the risk groups and implementing preventive interventions.

CONCLUSION

The results, obtained in the study, suggest that the investigated SNPs: G934T of *CPA4* gene, A19G of *LEP* gene and C-106T of *AKR1B* gene may not play a major role in the development and progression of chronic nephropathies.

Author's contributions

M.Ś. – participated in the design and performance of the study; J.G. – participated in the design and performance of the study; W.T. – participated in performance of the study; K.N. – participated in manuscript writing; W.G. – participated in the design of the study.

All authors read and approved the final manuscript.

REFERENCES

1. Collins A.J., Foley R.N., Chavers B. et al. United States Renal Data System 2011 Annual Data Report: Atlas of chronic kidney disease & end-stage renal disease in the United States. Am. J. Kidney Dis. 2012; 59(1 Suppl 1): A7, e1–420.

2. Coresh J., Selvin E., Stevens L.A. Prevalence of Chronic Kidney Disease in the United States. JAMA 2007; 298: 2038––2047.

3. Satko S.G., Sedor J.R., Iyengar S.K., Freedman B.I. Familial clustering of chronic kidney disease. Sem. Dial. 2007; 20: 229–236.

4. Cargill M., Altshuler D., Ireland J. et al. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat. Genet. 1999; 22: 231–238.

5. Spielman R.S., Ewens W.J. A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. Am. J. Hum. Genet. 1998; 62: 450–458.

6. Spielman R.S., McGinnis R.E., Ewens W.J. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am. J. Hum. Genet. 1993; 52: 506–516.

7. Poggio E.D., Wang X., Greene T., Van Lente F., Hall P.M. Performance of the Modification of Diet in Renal Disease and Cockcroft-Gault Equations in the Estimation of GFR in Health and in Chronic Kidney Disease. J. Am. Soc. Nephrol. 2005, 16: 459–466.

8. Schwartz G.J., Haycock G.B., Edelmann C.M., Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 1976; 58: 259–263.

9. Levey A.S., Eckardt K.U., Tsukamoto Y. et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 2005; 67: 2089–2100.

10. Laird N.M., Lange Ch. Family-based designs in the age of large-scale gene-association studies. Nat. Rev. Gen. 2006; 7: 385–394.

11. Placha G., Canani L.H., Warram J.H., Krolewski A.S. Evidence for different susceptibility genes for proteinuria and ESRD in type 2 diabetes. Adv. Chronic Kidney Dis. 2005; 12 (2): 155–169.

12. Wei S., Segura S., Vendrell J. et al. Identification and characterization of three members of the human metallo-carboxypeptidase gene family. J. Biol. Chem. 2002; 277: 14954–14964.

13. Huang H., Reed Ch.P. i wsp. Carboxypeptidase A3 (CPA3): a novel gene highly induced by histone deacetylase inhibitors during differentiation of prostate epithelial cancer cells. Cancer Res. 1999; 59: 2981–2988.

14. Pallares I., Bonet R., Garcia-Castellanos R. i wsp. Structure of human carboxypeptidase A4 with its endogenous protein inhibitor, latexin. PNAS. 2005; 102, 11: 3978–3983.

15. Hutchison F.N., Martin V.I. Effects of modulation of renal kallikreiny-kinin system in the nephrotic syndrome. Am. J. Physiol. 1990; 258: F1237–F1244.

16. Yu H., Bowden D.W., Spray B.J., Rich S.S., Freedman B.I. Identification of human plasma kallikrein gene polymorphism and evaluation of their role in end-stage renal disease. Hypertension 1998; 31: 906–911.

17. Mathew A.V., Okada S., Sharma K. Obesity related kidney disease. Curr. Diabetes Rev. 2011; 7: 41–49.

18. Papafraghaki D.K., Tolis G. Obesity and renal disease: a possible role of leptin. Hormones 2005; 4: 90–95.

19. Briley L.P., Szczech L.A. Leptin and renal disease. Sem. Dial. 2006; 19: 54–59.

20. Praga M., Hernandez E., Morales E. et al. Clinical features and long-term outcome of obesity-associated focal segmental glomerulosclerosis. Nephrol. Dial. Transplant. 2001; 16: 1790–1798.

21. Saxena A.K., Chopra R. Renal risk of an emerging „epidemic" of obesity: the role of adipocyte-derived factors. Nephrol. Dial. Transplant. 2004; 33, 1: 11–20.

22. Tutle K.R.: Renal manifestations of the metabolic syndrome. Nephrol. Dial. Transplant. 2005; 20: 861–864.

23. Han D.C., Isono M., Chen S. et al. Leptin stimulates type I collagen production in db/db mesangial cells: glucose uptake and TGF-beta type II receptor expression. Kidney Int. 2001; 59: 1315–1323.

24. Wolf G., Chen S., Han D.Ch., Ziyadeh F.N. Leptin and renal disease. Am. J. Kidney Dis. 2002; 39,1: 1–11.

25. Wolf G., Hamann A., Han D.C. et al. Leptin stimulates proliferation and TGF-β expression in renal glomerular endothelial cells: potential role in glomerulosclerosis. Kidney Int. 1999; 56: 860–872.

26. Carlyle M., Jones O.B., Kuo J.J., Hall J.E. Chronic cardiovascular and renal actions of leptin. Role of adrenergic activity. Hypertension 2002; 39 [part 2]: 496–501.

27. Kuo J.J., Jones O.B., Hall J.E. Inhibition of NO synthesis enhances chronic cardiovascular and renal actions of leptin. Hypertension 2001; 37 [part 2]: 670–676.

28. Granier C., Makni K., Molina L., Jardin-Watelet B., Ayadi H., Jarraya F.: Gene and protein markers of diabetic nephropathy. Nephrol. Dial. Transplant. 2008; 23: 792–799.

29. Shah V.O., Scavini M., Nikolic J. et al. Z-2 microsatellite allele is linked to increased expression of the aldose reductase gene in diabetic nephropathy. J. Clin. Endocrinol. Metab. 1998; 83: 2886–2891.

30. Makiishi T., Araki S., Koya D., Maeda S., Kashiwagi A., Haneda M. C-106T polymorphism of AKR1B1 is associated with diabetic nephropathy and erythrocyte aldose reductase kontent in Japanese subjects with type 2 diabetes mellitus. Am. J. Kidney Dis. 2003; 42: 943–951.

31. Moczulski D.K., Scott L., Antonellis A. et al. Aldose reductase gene polymorphisms and susceptibility to diabetic nephropathy in Type 1 diabetes mellitus. Diabet. Med. 2000; 17: 111–118.

32. Neamat-Allah M., Feeney S.A., Savage D.A. et al. Analysis of the association between diabetic nephropathy and polymorphism in the aldose reductase gene in type 1 and type 2 diabetes mellitus. Diabet. Med. 2001; 18: 906–914.

33. Wang Y., Ng M.C., Lee S.C. et al. Phenotypic heterogeneity and associations of two aldose reductase gene polymorphisms with nephropathy and retinopathy in type 2 diabetes. Diabetes Care 2003; 26: 2410–2415.

34. Dyer P.H., Chowdhury T.A., Dronsfield M.J., Dunger D., Barnett A.H., Bain S.C. The 5'-end polymorphism of the aldose reductase gene is not associated with diabetic nephropathy in Caucasian type I diabetic patients. Diabetologia 1999; 42: 1030–1031.

35. Ewens K.G., George R.A., Sharma K., Ziyadeh F.N., Spielman R.S. Assessment of 115 candidate genes for diabetic nephropathy by transmission/disequilibrium test. Diabetes. 2005; 54: 3305–3318.

36. Wolford J.K., Yeatts K.A., Red Eagle A.R., Nelson R.G., Knowler W.C., Hanson R.L. Variants in the gene encoding aldose reductase (AKR1B1) and diabetic nephropathy in American Indians. Diabet. Med. 2006; 23: 367–376.