Risk factors and activity markers in diabetes mellitus and proteinuria

Ph.D. thesis

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LIST OF ABBREVIATIONS

AGE	advanced glycation endproduct
BMI	body mass index
CIMT	carotis intima media thickness
FN3K	fructosamine-3-kinase
GCK	glucokinase
GCKR	glucokinase regulatory protein
GFR	glomerular filtration rate
HDL	high density lipoprotein
LDH	lactate-dehydrogenase
LDL	low density lipoprotein
MDRD	Modification Diet in Renal Deseases
NEG	non-enzymatic glycation
PCR	polimerase chain reaction
RFLP	restriction fragment length polymorphism
SNP	single nucleotide polymorphism
T2DM	type 2 diabetes mellitus

1. INTRODUCTION

Accelerated population ageing and urbanization, growing prevalence of obesity dramatically increased the incedence of type 2 diabetes mellitus (T2DM).

In the year 2000, 170 million people suffered in T2DM worldwide, and according the latest estimations, this is expected to double by the year 2030, reaching the number of 360 million patients. Diabetic micro- and macrovascular complications are significant economic burden to health care systems, therefore diabetes management should focus on preventing or delaying the onset of these complications.

Diabetic nephropathy is one of most dreaded microvasular complication of diabetes mellitus, subjects with diabetic nephropathy have four-fold higher cardiovasular risk compared with subjects without diabetic nephropathy.

Bad glycemic control, oxidative stress and the formation of advanced glycation endproducts (AGE) have a pivotal role in the pathogenesis. Among many others, genetic susceptibility is one of the most relevant risk factor. The genetic background of T2DM is complex and details yet remained unclarified. Several genes are involved in the pathogenesis, the genetic susceptibility can be determined as interaction of each diabetes genes, substantially influenced by the environmental factors and ethnicity.

Non-enzymatic glycation, is a process in which sugars react spontaneously with side chains of intra- and extracellular proteins ultimately leading through a series of further reactions to the formation of AGEs, which have crucial role in the development of diabetic microvascular complications.

AGEs are accumulated in tissues by ’in situ’ formation or via circulation. Incomplete detoxification of AGEs by the monocyte/macrophage system produces still toxic, low-molecular weight fragments, which can be eliminated only through the kidneys. During the elimination, AGE fragments bind to specific AGE receptors of the proximal tubular epithel cells and trigger inflammation ultimately leading to renal damage and the onset of albuminuria (diabetic nephropathy). In severe cases, proteinuria can reach the nephrotic range, which can cause ’per se’ renal damage. Therefore a vicious circle might develop.

There are several hypothesis as to how proteinuria, renal damage and atherosclerosis might be in strong association. Some say that proteinuria is a marker of renal damage and endothel dysfunction, in others oppinion proteinuria is not only a ’marker’, but the ’maker’ as well.

Filtrated protein is reabsorbed by the proximal tubular cells.In heavy proteinuria tubular cells get exhausted and damaged,cytokines induce inflammation resulting in tubulointerstitial fibrosis and ultimately end stage renal disease.

Serum total lactate-dehydrogenase (LDH) activityis a commonly used laboratoryparameter in the daily clinicalroutine. When cells are injured the enzyme is released into the bloodstream. Measuring the enzyme activity and the determination of isoenzyme pattern is highly informative in several diseases. However, there isn't any clinical laboratory test to clarify the etiology of nephrotic syndrome or to predict the prognosis.

Non-enzymatic glycation has been considered irreversible until a new intracellular enzymatic deglycation mechanism was discovered. A recently identified enzyme, namely the fructoseamine-3-kinase (FN3K) is considered as a potential protein repair enzyme, responsible for deglycation of intracellular proteins.It is able to phosphorylate low molecular mass fructosamines (i.e. fructoselysine) and other protein-bound fructosamines on the third carbon, resulting in an unstable compound, which then decomposes spontaneously to inorganic phosphate, to 3-deoxyglucasone and to the unmodified protein. The FN3K gene is located on chromosome 17q25, composed of 6 exons and encodes 309 amino acids. This 35 kDa monomeric enzyme is expressed constitutively in all tissues, however higher expression is observed in tissues exposed to extreme glycation, such as peripheral and central nervous system, kidneys, heart and in human erythrocytes. The role of FN3K was also verified in animal model, FN3K -/- mice have 2.5 fold higher level of intracellular glycated protein than in control mice.

Glucokinase (GCK) is a predominant glucose phosphorylating enzyme expressed in the liver and in the beta-cells of the Langerhans islets, playing a pivotal role in the glucose-stimulated insulin release as a physiological glucose-sensor. Pancreatic islets and the liver contain a regulatory protein (glucokinase regulatory protein, GCKR), which inhibits GCK in an allosteric manner with respect to glucose concentration byforming an inactive heterodimer. The inhibitory effect of GCKR is enhanced by fructose-6-phosphate and antagonized by fructose-1-phosphate. The 27 kb GCKR gene is located on chromosome 2p23 containing 19 exons and encodes a 68 kDa protein.

Genomewide association studies showed, that common functional variants of the GCKR gene are associated with fasting plasma glucose and serum triglycerides. More recently, both functional variants of the GCKR gene were widely investigated as candidate T2DM susceptibility variants, and susceptibility genes for hypertigliceridemia.

Hypertriglyceridemia confers an independent significant risk for cardiovascular diseases, accordingly studying the possible association of rs780094 and rs1260326 of the GCKR gene on metabolic risk traits might have importance in cardiovascular diseases.

Taking all this together; association studies of SNPs influencing the lipid traits and the AGE level have a capital importance in the risk assessment of diabetic (diabetic nephropathy) and non-diabetic kidney diseases.

2. AIMS OF THE STUDY

1. Determination the allelic distribution and association study of the SNP G900C (rs1056534) of the FN3K gene in T2DM patients and in healthy control subjects. Study the possible protective nature against diabetic microvascular complications particularly against diabetic nephropathy.

2. Determination the allelic distribution and association study of the functional variants of the GCKR gene (rs780094, rs1260326) on carbohydrate and lipid metabolism and the carotid intima-media thickness (CIMT) in T2DM patients, in metabolic syndrome patients and in healthy control subjects. Study the possible susceptibility or protective nature against diabetic microvascular complications particularly against diabetic nephropathy.

3. Study the LDH activity and LDH isoenzyme pattern and the prognostic value of LDH in nephrotic syndrome and in non-nephrotic syndrome patients.

3. MATERIALS AND METHODS

3. 1. Study population

All patients were enrolled from the 2nd Department of Medicine and Nephrological Center, University of Pécs, Hungary.T2DM was diagnosed according to the criteria of the World Health Organization. Metabolic syndrome was diagnosed according to modified criteria of the Adult Treatment Panel III of National Cholesterol Education Program.
Controls were gathered from trauma units, blood donors, medical staff and university students, they were free from any single clinical or laboratory sign of T2DM or metabolic syndrome; their medical history were also free from any systemic or organ-specific disease. All participants gave their informed consent and the study was approved by the local Ethics Committee.

3. 2. Biochemical and clinical data

All participants underwent a detailed medical examination. Laboratory parameters were assessed using routine methods from fasting blood samples. BMI was calculated as weight (kg) divided by height (m2). Glomerular filtration rate (GFR) was estimated by the Modification Diet in Renal Deseases (MDRD) formula. CIMT was measured by B-mode ultrasound device with a high resolution 10 MHz linear transducer. LDH isozymes were separated by agarose gel electrophoresis and the proportions of the enzymes were determined by their activity with densitometry.

3. 3. Genotyping

DNA samples and the clinical data were deposited into the Central Biobank governed by the University of Pécs, as part of the National Biobank Network of Hungary, approved by the national Scientific Research Ethics Committee. Genotyping was carried out at the Department of Medical Genetics, Faculty of Medicine, University of Pécs.

DNA was isolated from peripheral blood leukocytes by standard salting out method. Genotyping was determined with polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method.

The FN3K G900C polymorphism site was amplified using the primers 5'- GGT TTC CCC AGA TCC TTC TTC (forward) and 5'- GAC AGG GGG ATT GGT ATG TG (reverse).The 400 bp amplicon was digested with Eco130I (StyI) restriction enzyme; after digestion 139, 261 bp fragments were in the samples with GG genotype, in homozygous CC samples 43, 96, 261 bp products were detected. For amplification PTC 200 PCR equipments were used, the conditions were similar for all polymorphisms: a 2 min initial denaturation at 96 ^{o}C was followed by 35 cycles of 20 sec at 96 ^{o}C; 20 sec at 60 ^{o}C; and 20 sec at 72 ^{o}C; the final extension occurred at 72 ^{o}C for 5 min. The amplification was carried out in a final volume of 50 µl containing 5 µl reaction buffer (500 mM KCl, 14 mM $MgCl_2$, 10 mM Tris-HCl, pH 9.0), 1 µl 50 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U of Taq polymerase, 0.2 mM of the adequate specific primer pairs and 1 µg DNA.

By GCKR gene polymorphism, for PCR amplification, the following primers were used: GCKR rs1260326: forward 5'-TGC AGA CTA TAG TGG AGC CG-3' and reverse 5'-CAT CAC ATG GCC ACT GCT TT-3'; GCKR rs780094: forward 5'-GAT TGT CTC AGG CAA ACC TGG TAG-3' and reverse 5'-CTA GGA GTG GTG GCA TAC ACC TG-3'. An MJ Research PTC-200 thermal cycler was used for the amplification. PCR conditions were the following: predenaturation at 96°C for 2 min; followed by 35 cycles of denaturation at 96°C for 20 sec (rs1260326), 30 cycles of denaturation at 96°C for 20 sec (rs780094); annealing at 60°C for 20 sec (rs1260326), and at 62°C for 30 sec (rs780094); primer extension for 30 sec at 72°C; and final extension at 72°C for 5 min. The amplicons were digested by HpaII restriction endonuclease (rs1260326) and PscI (rs780094). The digestion of 231 bp amplicon of rs1260326 CC genotype resulted in 18, 63, 150 bp fragments; the TT genotype 18 and 213 bp; while the heterozygous genotype 18, 63, 150, 213 bp fragments. For the rs780094 427 bp amplicon the following fragments were detected: GG genotype 62, 177, 188 bp; AA genotype 62, 365 bp; heterozygous genotype 62, 177, 188, 365 bp fragments.

3. 4. Statistical analysis

All statistical procedures were performed using the SPSS 15.0 software. P values ≤ 0.05 were considered statistically significant. Kolmogorov-Smirnov test was used to assess sample distribution. Clinical and biochemical characteristics of the study participants were compared with one-way ANOVA, with analysis of covariance (ANCOVA) and with Kruskal-Wallis followed by Mann-Whitney U test. To test for correlation, we used the Pearson's and the the Spearman's rho test, according to the distribution. A stepwise linear regression was carried

out to determine the independent predictors. Survival curves were calculated using Kaplan-Meier method and the curves were compared by log-rank test.Chi-square test was used to compare the qualitative data. Multivariate logistic regression analysis models were used to risk evaluation.

4. RESULTS AND DISCUSSION

4. 1. Studying the fructosamine-3-kinase G900C polymorphism

Recent studies revealed an enzyme, called FN3K, which can protect proteins against NEG. Several SNPs of the FN3K gene were identified, among which the CC allelic variant of the G900C SNP was associated inversely with the enzyme activity. In this study we investigated the possible association between clinical parameters and the presence of late diabetic complication and the G900C polymorphism of FN3K genein type 2 diabetic subjects.

The genotype frequencies were as follows, GG: 41%, GC: 54%, CC: 5% in subjects with T2DM and GG: 43%, GC: 51%, CC: 6% in the controls, and followed the Hardy-Weinberg equilibrium. We did not observe significant differences in the genotype frequencies between the groups.

Several reports suggested the potencial association between FN3K activity and the glycated haemoglobin level, however, they failed to detect correlation between SNPs of FN3K and the HbA_{1c} levels. On the contrary, we found significantly decreased HbA_{1c} levels in T2DM subjects with the CC genotype compared with CG and GG variants (CC: 6.48±0.05%; GC: 7.66±0.09%; GG: 7.68±0.09%, $p<0.001$). Subjects with the highest HbA_{1c} level (3. tertile) had significantly lower frequency of the C allele compared with subjects with the lowest HbA_{1c} levels (1. tertile) (1. tertile: 34.3% vs. 3. tertile: 29.0, $p<0.05$).

Furthermore, we found association between the polymorphism and the onset of disease, in case of the CC allelic variant T2DM was diagnosed at a later age than in case of GC or GG variants ($p<0.05$).

Some studies reported association between FN3K activity and the diabetic microvscular complications. According the latest observations, in diabetic nephropathy the level of FN3K mRNA was significantly lower in the progressors compared with the non-progressors, suggesting the prognostic effect of the enzyme.

Logistic regression analysis revealed that the CC variant was not associated with the diabetic microvascular complications (diabetic nephropathy, neuropathy and retinopathy), adjusted for age, gender, BMI, duration and onset of diabetes (OR=1.036, CI 95% 0.652-1.647, p=0.880; OR=0.985, CI 95% 0.564-1.721 p=0.958; OR=1.213, CI 95% 0.470-3.132, p=0.690, respectively).

The analyzed SNP does not induce amino acid change; moreover it is present neither in sequences of splicing sites nor in binding sites of transcription factors, so the altering effect

on enzyme activity of the SNP is not fully elucidated. It might presumably be due to sequence variations in or near the FN3K gene, or due to the linkage disequilibrium with the A-385G polymorphism of the promoter region of the FN3K gene, or with other regulatory elements; however other mechanisms cannot be ruled out.

4. 2. Studying the GCKR functional gene variants

Recently, GCKR gene were widely investigated as candidate T2DM susceptibility gene. Genomewide association studies showed, that common functional variants of the GCKR gene (rs1260326, rs780094) are associated with triglycerides. Other studies revealed association with the fasting plasma glucose, insulin levels. Inverse effect on triglycerides and fasting plasma glucose concentration was also confirmed in different populations. In SNP rs1260326 of the GCKR a C/T change results in a proline to leucine substitution in the aminoacid sequence of the encoded protein. This change is very likely to modify the structure of the protein and if this structural alteration is in the binding site of fructose-6-phosphate or fructose-1-phosphate it can influence the function of the protein. GCKR rs1260326 variant (P446L) is in strong linkage disequilibrium (r2 = 0.96) with rs780094 variant.

In this study, we investigated the functional variants of the GCKR gene (rs780094, rs1260326) in T2DM patients, in metabolic syndrome patients and in healthy control subjects.

In the current study, we could replicate the previously reported positive associations of the two functional variants of GCKR gene (rs780094 and rs1260326) and triglyceride/glucose metabolism in the Hungarian population; our results confirmed the inverse association with serum triglycerides and plasma glucose levels in T2DM and metabolic syndrome patients.

The genotype profiles including the minor allele frequencies were similar in the study groups (rs780094: A allele, controls: 47.4%, T2DM: 46.9%, metabolic syndrome: 47.6%, $p>0.05$; rs1260326: T allele, controls: 48.8%, T2DM: 47.1%, metabolic syndrome: 46.8%, $p>0.05$). All genotypes were in Hardy-Weinberg equilibrium.

Body mass index, fasting plasma glucose, lipid profile and CIMT in subjects with metabolic syndrome, T2DM and controls by individual genotypes are presented in *table A and B.*

BMI is not associated either with rs780094 or with rs1260326, however plasma glucose levels were associated with both variants significantly ($p<0.05$). We observed association of the minor allele of rs780094 and rs1260326 with elevated serum triglycerides in all groups. We found no relationship between variants of GCKR gene and total serum cholesterol levels, however HDL-cholesterol level was significantly lower in subjects homozygous for the minor

allele for both rs780094 and rs1260326, but only in T2DM patients, moreover LDL-cholesterol was significantly elevated in homozygous patients, but only in the metabolic syndrome group. We also correlated the CIMT, the subclinic marker of atheroslerosis in patients with T2DM and metabolic syndrome. Both variant was associated with increased CIMT.

Subjects with the highest glucose level(3. tertile) had significantly lower frequency of the minor A and T allele compared with subjects with the lowest glucose level (1. tertile) and similarly subjects with the highest triglycerides(3. tertile)had significantly higher frequency of the minor A and T allele compared with subjects with the lowest triglycerides($p<0.05$).

Regression analysis revealed that both rs780094 and rs1260326 confer a significant susceptibility for the development of hypertriglyceridemia; after adjusting the results for age, gender, total serum cholesterol, the presence of coronary artery disease and statin therapy (rs780094, OR (CI 95%):1.748(1.256-2.435), p=0.001; rs1260326, OR (CI 95%): 1.311(1.078-1.596), p=0.007).

None of the variants were associated with the development of T2DM and metabolic syndrome or with the diabetic microvascular complications (diabetic nephropathy, neuropathy and retinopathy) by logistic regression analysis.

GCKR plays an important role in the carbohydrate and lipid metabolism. There are some hypotheses for the mechanism of action, however its physiological role has not been clarified yet.

4. 3. 3. Study the LDH activity and LDH isoenzyme pattern and the prognostic value of LDH in nephrotic syndrome and in non-nephrotic syndrome patients.

Measuring the serum total LDH activity is an important diagnostic parameter in different diseases with tissue-damage because during cell-lesion this enzyme is released into the circulation [8]. Determination of LDH isozyme pattern is a great assistance in elucidating the origin of the enzyme. The role of serum total LDH activity in different kidney diseases has been studied only in few studies so far.

In nephrotic syndrome the filtrated proteins overflow the proximal tubules where tubular cells reabsorb proteins by endocytosis. Since this is a high energy-demanding procedure, tubular cells may get exhausted and damaged soon. Large quantities of proteins have a toxic effect on tubular cells by increasing the cell proliferation and apoptosis thus the tubular cell turnover accelerates. Glomerular barrier protects normally serum proteins from filtration into the urine.

Presumably, in nephrotic syndrome some serum proteins (e.g. albumin and transferrin) get into the proximal tubules and induce inflammation resulting in damage of apical and basolateral membrane of the epithelial cells, therefore LDH can freely flow out from the cytoplasm into the serum and urine.

We studyed the LDH activity and LDH isoenzyme pattern and the prognostic value of LDH in nephrotic syndrome (NEPHR) and in non-nephrotic syndrome patients (NON-NEPHR) and in hypoalbuminemic subjects with different gastrointestinal diseases as a positive control group (KONTR).

Serum total LDH activity of the NEPHR group was significantly higher compared with the NON-NEPHR and KONTR groups ($p<0.001$). LDH isoenzyme patterns were determined in three randomly selected subgroups.

Both the activity and the ratio of kidney specific LDH-2 were significantly higher in the nephrotic subgroup compared with the non-nephrotic and control subgroups ($p<0.001$).

The elevation of serum total LDH activity in nephrotic patients is not a marker of retention.We investigated the serum total LDH activity (prior to dialysis) in hemodialysis patients and found that the serum total LDH activity was not significantly different compared with the NEPHR group (308±7 U/l, $p=0.703$)and did not differ from the NON-NEPHR and CONTR groups.

Serum total LDH activity correlated negatively with the serum total protein and serum albumin level ($r= -0.549$ $p<0.001$; $r=-0.596$ $p<0.001$) and positively with proteinuria and serum total cholesterol level ($r=0.456$ $p<0.001$; $r=0.523$, $p<0.001$).

Serum LDH-2 activity was found to correlate negatively with the serum total protein and serum albumin level and positively with proteinuria and serum total cholesterol level. We analyzed the correlations with the ratio of serum LDH-2 as well, and we found that it also correlated negatively with serum total protein and serum albumin level ($r= -0.607$, $p<0.001$; $r= -0.482$, $p<0.001$), and positively with proteinuria and serum total cholesterol level ($r=0.658$, $p<0.001$; $r=0.648$, $p<0.001$).

As a result of the linear regression analysis, serum total protein level proved to be independent predictor of serum total LDH activity ($B=-5.831$ $\beta=-0.648$ $p<0.001$) while serum total protein and proteinuria were verified as predictors of LDH-2 ($B=-0.273$ $\beta=-0.457$ $p=0.007$; $B=1.121$, $\beta=0.428$, $p=0.011$).

During a 300 weeks follow-up period, renal survival curves of nephrotic syndrome patients did not differ significantly between patients with LDH above and below the median LDH value ($p=0.196$).

We conclude, that LDH is an activity marker in nephrotic syndrome and together with isoezyme analysis might give important information about the activity and severity of the disease.

Table A. Body mass index, fasting plasma glucose, lipid profile and carotid intima-media thickness in subjects withmetabolic syndrome, type 2diabetes mellitus and controls by individual genotypes

		Control			T2DM			MS		
		GG	GA	AA	GG	GA	AA	GG	GA	AA
		n=44	n=93	n=35	n=77	n=151	N=78	n=121	n=217	n=100
GCKR (rs780094)	BMI (kg/m^2)	23.6 (21.2-28.6)	23.9 (15.8-32.8)	23.8 (13.7-26.8)	29.2 (17.0-48.6)	29.0 (18.3-45.9)	27.3 (18.3-43.4)	32.5 (22.5-52.5)	32.7 (20.4-48.1)	32.9 (19.4-60.5)
	Fasting plasma glucose (mmol/l)	N/A	N/A	N/A	9.50 (3.00-17.0)	9.20 (3.80-22.8)	8.90 (2.30-17.1)*	9.60 (3.80-19.1)	8.80 (3.00-30.8)	8.50 (4.51-16.6)*
	Serum triglycerides (mmol/l)	1.35 (0.50-2.90)	1.50 (0.80-3.60)	1.70 (0.70-3.20)*	1.77 (0.38-8.23)	1.81 (0.35-6.22)	2.24 (0.66-14.8)*	2.07 (0.49-7.76)	2.68 (0.35-14.2)*	3.07 (0.78-12.3)#
	Serum total-cholesterol (mmol/l)	5.48±0.80	5.44±0.94	5.04±1.04	5.01±1.02	4.60±1.12	4.75±1.10	5.03±1.05	5.15±1.36	5.35±1.26
	Serum HDL-cholesterol (mmol/l)	N/A	N/A	N/A	1.39 (0.76-2.48)	1.25 (0.68-2.37)	1.19 (0.62-2.49)*	1.21 (0.77-1.99)	1.22 (0.55-2.12)	1.17 (0.79-1.88)
	Serum LDL-cholesterol (mmol/l)	N/A	N/A	N/A	2.84±0.90	2.64±0.83	2.75±0.89	2.08±0.81	2.70±0.83	3.06±0.92*
	CIMT (mm)	N/A	N/A	N/A	0.81±0.42	0.86±0.38	0.95±0.44	0.79±0.28	0.87±0.32	1.06±0.26*

** p < 0.05 vs. GG; # p < 0.001 vs. GG; BMI, body mass index; CIMT, carotid intima-media thickness; HDL, high-density lipoprotein;LDL, low-density lipoprotein; MS, metabolic syndrome; T2DM, type 2 diabetes mellitus; Data are mean ± SD or median (minimum-maximum) as appropriate*

Table B. Body mass index, fasting plasma glucose, lipid profile and carotid intima-media thickness in subjects withmetabolic syndrome, type 2diabetes mellitus and controls by individual genotypes

		Control			T2DM			MS		
		CC	TC	TT	CC	TC	TT	CC	TC	TT
		n=48	n=80	n=44	n=80	n=155	n=63	n=118	n=219	n=91
GCKR (rs1260326)	BMI (kg/m^2)	23.7 (20.8-28.6)	23.8 (15.8-32.8)	23.8 (13.7-26.8)	29.2 (17.0-37.6)	29.3 (18.3-45.9)	28.9 (18.3-38.6)	31.8 (20.4-49.57)	32.8 (19.4-52.5)	33.4 (25.0-60.5)
	Fasting plasma glucose (mmol/l)	N/A	N/A	N/A	9.05 (3.00-16.9)	8.90 (2.30-17.1)	8.70 (2.30-22.8)*	9.80 (3.20-20.0)	8.60 (3.90-30.8)	8.40 (3.00-16.6)*
	Serum triglycerides (mmol/l)	1.51 (0.50-2.90)	1.55 (0.70-2.90)	1.68 (0.80-3.60)*	1.45 (0.35-4.28)	1.87 (0.39-8.23)*	2.32 (0.59-14.9)*	2.19 (0.70-7.86)	2.72 (0.35-14.4)*	2.91 (0.78-11.5)*
	Serum total-cholesterol (mmol/l)	5.44±0.87	5.41±0.96	5.20±0.99	5.03±1.11	4.64±0.97	4.72±1.32	5.10±1.02	5.21±1.38	5.20±1.27
	Serum HDL-cholesterol (mmol/l)	N/A	N/A	N/A	1.39 (0.76-2.48)	1.27 (0.68-2.47)	1.17 (0.62-2.49)*	1.23 (0.70-1.96)	1.16 (0.55-2.12)	1.23 (0.79-1.88)
	Serum LDL-cholesterol (mmol/l)	N/A	N/A	N/A	2.85±0.88	2.63±0.78	2.78±1.05	2.71±0.81	2.85±0.82	3.00±0.93*
	CIMT (mm)	N/A	N/A	N/A	0.81±0.41	0.88±0.43	0.95±0.44	0.83±0.30	0.87±0.38	1.05±0.28*

** p<0.05 vs. CC; BMI, body mass index; CIMT, carotid intima-media thickness; HDL, high-density lipoprotein;LDL, low-density lipoprotein; MS, metabolic syndrome; T2DM, type 2 diabetes mellitus; Data are mean ±SD or median (minimum-maximum) as appropriate*

5. THESES

1. G900C polymorphism of FN3K gene is associated with the HbA_{1c} level, with the duration of T2DM and with the age at onset of T2DM, but not with the diabetic microvascular complications.

2. In T2DM, functional variants of the GCKR gene (rs780094, rs1260326) are associated with serum triglycerides, with the fasting plasma glucose level, with the HDL-cholesterol level and CIMT. In metabolikuc syndrome,both functional variants are associated with serum triglycerides, with the fasting plasma glucose level, with the LDL-cholesterol level and CIMT. None of the variants are associated with the diabetic microvascular complications.

3. Serum LDH and LDH-2 isoenzyme correlates with routine laboratory parameters of nephrotic syndrome. Serum LDH and LDH-2 isoenzyme is an activity marker, but not a prognostic marker of nephrotic syndrome.

6. LIST OF PUBLICATIONS

The thesis is based on the following publications

1. **Mohás M**, Markó L, Molnár GA, Cseh J, Laczy B, Tamaskó M, Balla J, Kappelmayer J, Wagner L, Wagner Z, Csiky B, Nagy J, Wittmann I: A szérum-LDH-aktivitás és a vese-LDH izoenzim jelentősége nephrosis szindrómában. *Magyar Belorv Arch* 2007;62:47-52.

2. **Mohás M**, Szigeti N, Markó L, Molnár GA, Cseh J, Laczy B, Tamaskó M, Balla J, Kappelmayer J, Wagner L, Wagner Z, Csiky B, Nagy J, Wittmann I: Serum total LDH activity and LDH-2 isozyme in nephrotic syndrome. *Kidney Blood Press Res* 2008;31(1):47-54.

3. **Mohás M**, Kisfali P, Baricza E, Mérei Á, MaászA, Cseh J, Mikolás E, Szijártó IA, Melegh B, Wittmann I: A polymorphism within the fructosamine-3-kinase gene is associated with HbA_{1c} levels and the onset of type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*2010;118(3):209-212.

4. **Mohás M**,Kisfali P, Járomi L,Maász A, Fehér E, Csöngei V, Polgár N, Sáfrány E, Cseh J, Sümegi K, Hetyésy K, Wittmann I, Melegh B: GCKR gene functional variants in type 2 diabetes and metabolic syndrome: do the rare variants associate with increased carotid intima-media thickness? *Cardiovasc Diabetol*2010;29(9):79.

5. **Mohás M**, Kisfali P, Baricza E, Mérei Á,Duga B, Maász A, Cseh J, Mikolás E, Szijártó I, Melegh B, Wittmann I: A fruktózamin-3-kináz enzim C900G gén polimorfizmusának vizsgálata 2-es típusú cukorbetegekben. *Diabetologica Hungarica* 2011;19(1):43-48.

Other publications

1. Tamaskó M, Kalmár Nagy K, Degrell P, Molnár GA, Dérczy K, Schmidt E, Kalabay L, Wagner L, Wagner Z, Mazak I, Laczy B, Markó L, **Mohás M**, Nagy J, Wittmann I:

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