Fructosamine 3-Kinase and Glyoxalase I Polymorphisms and Their Association With Soluble RAGE and Adhesion Molecules in Diabetes

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Summary

Advanced glycation end-products (AGEs) are key players in pathogenesis of long-term vascular diabetes complications. Several enzymes such as fructosamine 3-kinase (FN3K) and glyoxalase I (GLO I) are crucial in preventing glycation processes. The aim of our study was to evaluate an association of *FN3K* (rs1056534, rs3848403) and *GLO1* rs4746 polymorphisms with parameters of endothelial dysfunction and soluble receptor for AGEs (sRAGE) in 595 diabetic and non-diabetic subjects. Genotypic and allelic frequencies of mentioned polymorphisms did not differ between subgroups. In diabetic patients significant differences were observed in sRAGE concentrations according to their rs1056534 and rs3848403 genotype. While GG and CG genotypes of rs1056534 with mutated G allele were associated with significant decrease of sRAGE (GG: 1055±458 and CG: 983±363 vs. CC: 1796±987 ng/l, $p<0.0001$), in rs3848403 polymorphism TT genotype with mutated T allele was related with significant sRAGE increase (TT: 1365±852 vs. CT: 1016±401 and CC: 1087±508 ng/l, $p=0.05$). Significant differences in adhesion molecules were observed in genotype subgroups of *GLO1* rs4746 polymorphism. In conclusion, this is the first study describing significant relationship of *FN3K* (rs1056534) and (rs3848403) polymorphisms with concentration of sRAGE in patients with diabetes.

Key words

Fructosamine 3-kinase • Glyoxalase I • Diabetes • sRAGE • Adhesion molecules

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Introduction

Advanced glycation end-products (AGEs) are key players in the pathogenesis of long-term vascular complications in patients with diabetes (Brownlee 2005, Genuth *et al.* 2005, Vlassara and Striker 2013). AGEs originate by non-enzymatic glycation of proteins, forming cross-links with collagen and other proteins, resulting in decreased vessel elasticity (Zieman *et al.* 2007). One of the most potent precursor is methylglyoxal (MG), which causes irreversible effects on protein structure and function (Silva *et al.* 2013). Another important precursor for excessive glycation is fructosamine (Popova *et al.* 2010). Apart from endogenously produced AGEs, the human body is also exposed to oral AGEs in food, which also further promotes insulin resistance and diabetes development (Cai *et al.* 2012). In this respect, deglycation processes are essential for deceleration of vascular damage progression. Two enzymatic systems rank among the most important protective factors – fructosamine 3-kinase and glyoxalase system.

Fructosamine 3-kinase (FN3K) is an intracellular enzyme responsible for deglycation of proteins. Higher

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expression of this enzyme was observed in increased-glycation-prone tissues, such as heart, nerves and kidneys (Mohas *et al.* 2010). Phosphorylation of fructosamine by FN3K leads to production of unstable fructosamine 3-phosphate, which further decomposes to 3-deoxyglucosone and phosphate, leading to deglycation of proteins (Delpierre and Van Schaftingen 2003). On the other hand, 3-deoxyglucosone is a potent precursor for AGEs formation, elevated in diabetic patients with microangiopathy (Kusunoki *et al.* 2003). The gene for FN3K is located on chromosome 17q25. So far, various polymorphisms of the gene have been described, some of them having impact on FN3K activity in human erythrocytes (Delpierre *et al.* 2006).

Glyoxalase I catalyzes acyclic alpha-oxoaldehydes conversion to hydroxyacylglutathione derivatives and thus prevents glycation reactions mediated by methylglyoxal, glyoxal and other alpha-oxoaldehydes. Decreased glyoxalase I activity caused by aging and oxidative stress results in increased glycation and tissue damage (Song and Schmidt 2012). Moreover, glyoxalase I deficiency is associated with an unusual level of advanced glycation end products in a hemodialysis patient (Miyata *et al.* 2001), while knockdown of glyoxalase I even mimics diabetic nephropathy in nondiabetic mice (Giacco *et al.* 2014). The gene encoding GLO I is located on chromosome 6p21.2. Several polymorphisms of *GLO1* were studied, most of the data describing *GLO1* rs4746 polymorphism, where wild type A allele is substituted by mutated C allele. Such polymorphism is associated with reduced activity of the enzyme, accumulation of its substrate methylglyoxal and elevation of RAGE in patients with autism (Barua *et al.* 2011). Similarly, *GLO1* rs4746 polymorphism was associated with elevated sRAGE levels in hemodialysis patients (Kalousova *et al.* 2008).

Higher concentration of different AGEs leads to increased binding to receptor for AGEs (RAGE) with subsequent acceleration of chronic inflammation performed by enhanced expression of genes for growth factors or cytokines (IFNγ, PDGF, TNFα, IL-1) and adhesion molecules (ICAM-1, VCAM-1, P-selectin, E-selectin). The involvement of RAGE and soluble RAGE (sRAGE) in the pathophysiology of diabetes angiopathy has been reported previously (Yan *et al.* 2007, Lindsey *et al.* 2009, Skrha *et al.* 2012).

The aim of our study was to investigate polymorphisms of genes encoding important enzymes involved in the deglycation systems (*FN3K* rs3848403, *FN3K* rs1056534 and *GLO1* rs4746) and their relationship to the development of chronic micro- and macrovascular damage in diabetes. Since the involvement of RAGE activation and endothelial dysfunction in the development of diabetic vascular damage seems to be of great importance (Roberts and Porter 2013), we also analyzed concentrations of sRAGE and markers of endothelial dysfunction in our patients.

Patients and Methods

Subjects

A total of 595 Caucasian subjects were enrolled in this study (311 men and 284 women), from those 126 healthy controls, 129 patients with Type 1 diabetes (T1DM) and 340 patients with Type 2 diabetes (T2DM). Their characteristics are shown in Table 1. All patients with severe hypertension, neurodegenerative disorders, known malignancy, or infections, which could significantly influence laboratory variables, were excluded from the study. The incidence of known microvascular and macrovascular complications among the subjects and basic pharmacological treatment of the patients is presented in Table 2.

The study was performed in accordance with principles of the Declaration of Helsinki and was approved by local Ethics Committee of the General University Hospital and First Faculty of Medicine. All examined persons gave informed consent prior to being enrolled into the study.

Biochemical methods

Fasting blood samples were collected between 7.00 and 8.00 AM from the cubital vein. Routine biochemical parameters were determined in fresh samples, whereas special biochemical analyses were done in serum frozen at −80 °C until the assay measurement. Routine biochemical parameters including urea, creatinine, transaminases, alkaline phosphatase, γ-glutamyltransferase, total cholesterol, and triglycerides were determined in central laboratory on Modular Roche analyzer. Fasting plasma glucose was determined by glucose oxidase method on glucose analyzer Super GLAmbulance (Dr. Müller Gerätebau, Freital, Germany); glycated hemoglobin HbA_{1c} was measured by HPLC on Variant II (Biorad, France) and expressed according to IFCC (normal values are 28-40 mmol/mol). Albuminuria was determined after exclusion of urinary infection by radioimmunoassay using commercial kits (Immunotech,

Czech Republic) and urinary albumin/creatinine ratio (ACR) was calculated. Presence of nephropathy was characterized by positive (micro)albuminuria, which was recognized by albumin/creatinine ratio >3 g/mol creatinine. Logarithmically transformed data were used for further analysis, because lognormal distribution of the values was found. Renal functions were evaluated by estimated glomerular filtration rate (eGFR) calculated by MDRD formula (Levey *et al.* 1999).

In 126 subjects (50 T1DM, 52 T2DM and 24 healthy controls; serum of other subjects was no more available) analysis of sRAGE and markers of endothelial activation was performed. Serum concentration of soluble RAGE was measured according to the manufacturer's protocol using sandwich ELISA (Quantikine, RD Systems, Minneapolis, MN, USA). In this assay, the plate is coated with a monoclonal antibody against RAGE while a polyclonal antibody is used for detection. This assay measures both C-truncated RAGE that has been cleaved from the cell surface, and esRAGE as well (Yonekura *et al.* 2003). Endothelial dysfunction was evaluated by serum concentrations of specific markers, such as adhesion molecules (ICAM, VCAM, E-selectin, and P-selectin) and vWF. Cell adhesion molecules Human sP-selectin/CD62P, Human sE-selectin/CD62E, Human sICAM-1/CD54, and Human sVCAM-1 were estimated by ELISA kits manufactured by RD System Europe (Abingdon, UK), von Willebrand factor (vWF) was determined by Corgenix (Broomfield, USA).

Molecular genotyping

For DNA analysis blood was collected into EDTA-tubes, centrifuged and stored at 4 °C until isolation. Genomic DNA was prepared from leukocytes by sodium dodecylsulphate (SDS) lysis by ammonium acetate extraction and ethanol precipitation. Isolated DNA was stored at 4 °C.

FN3K polymorphisms

Two SNPs of *FN3K* gene were studied for their potential functional effect on enzyme activity (rs1056534 (Ser300Ser, S300S) and rs3848403 (C/T intron variant). DNA analysis was performed using RealTime PCR and Taqman genotyping method for allelic discrimination. For quality control, the subjects were distributed randomly across the plates. Negative controls (Universal-mixture blanks) were included onto each plate. The genotyping success rate was 95 % (range 91 to 98 %).

GLO1 polymorphism

The *GLO1* rs4746 (Glu111Ala, A419C) polymorphism of the glyoxalase I gene was determined by PCR-RFLP method as described in detail previously (Kalousova *et al.* 2008, Germanova *et al.* 2009). Restriction analysis was performed by restriction nuclease BsmAI overnight at 37 °C. Fragments of 143 bp and 60 bp for wild type allele 419A and 203 bp for mutated allele 419C were produced after digestion.

Statistical analysis

Results of biochemical parameters were expressed as mean ± standard deviation (SD) or as median (interquartile range). Differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by *post-hoc* test analysis. The allelic frequencies for each polymorphism were calculated in all groups. χ^2 test was used to compare the qualitative data. Moreover, Hardy-Weinberg equilibrium (HWE) within the groups was estimated by χ^2 test as well. Results were considered statistically significant for p-values <0.05.

Results

Clinical characteristics of 595 subjects enrolled within this study are shown in Table 1. Diabetes control did not differ significantly between patients with T1DM and T2DM (FPG: 8.7±3.6 vs. 8.7±3.3 mmol/l, ns; HbA_{1c}: 69±14 vs. 64±21 mmol/mol, ns), although the duration of the disease was longer in T1DM (16 vs. 11 years, p<0.001). Incidence of both microvascular and macrovascular complications in patients with diabetes is shown in Table 2, as well as the usage of antidiabetic, antihypertensive and hypolipidemic drugs.

In both patients with T1DM, T2DM and controls the genotype frequencies of *FN3K* and *GLO1* polymorphisms followed the expected frequencies according to Hardy-Weinberg equilibrium (HWE). We did not find any significant differences of genotype and allelic frequencies of *FN3K* (rs1056534), *FN3K* (rs3848403) and *GLO1* (rs4746) polymorphisms within the studied groups (Table 3). Similarly, we did not observe differences of genotype and allelic frequencies in studied polymorphisms among patients with or without developed microvascular (nephropathy, neuropathy, retinopathy) and macrovascular (ischemic heart disease, chronic limb ischemia, stroke) complications.

Table 1. Basic clinical characteristics of studied subjects.

	T1DM (n=129)	T2DM (n=340)	Controls (n=126)	*ANOVA*
Age (years)	46 (20-80)	63 (26-93)	45 (18-91)	p<0.0001
Duration of DM (years)	16 (1-57)	11 (1-44)	-	
Sex (% males)	58	52	48	ns
BMI (kg/m^2)	25.0±3.1^z	29.5±5.9^c	25.4±4.8	p<0.005
SBP (mm Hg)	132±13	138±18	129±14	ns
DBP (mm Hg)	81±10	79±11	77±9	ns
Creatinine (μmol/l)	83.2±38.4^x	95.7±52.5^b	78.0±22.3	p<0.05
Cholesterol (mmol/l)	4.63±0.77	4.44±0.92	4.52±0.82	ns
Triglycerides (mmol/l)	1.03±0.49^z	1.79±1.17^c	1.05±0.61	p<0.0001
FPG (mmol/l)	8.7±3.6^c	8.7±3.3^c	4.4±0.8	p<0.0001
HbA$_{1c}$ (mmol/mol, IFCC)	69±14^c	64±21^c	37±13	p<0.0001
sRAGE (ng/l)	1083±420^a	1119±619^a	785±314	p<0.05
Albumin/creatinine (g/mol)	1.22^{bz} (0.1-22.5)	7.53^c (0.2-294.3)	0.48 (0.1-1.8)	p<0.0001

Results are means ± SD, or means with 1 SD range, in DM duration median with ranges. One-way ANOVA was performed, with p values in the last column of the table. Statistical significance expressed by LSD multiple comparison *post-hoc* test between DM and control persons: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, and between T1DM and T2DM: ^{x}p<0.05, ^{y}p<0.01, ^{z}p<0.001.

Table 2. Incidence of diabetic complications and frequency of antidiabetic/hypolipidemic/antihypertensive drugs usage.

	T1DM (n=129)	T2DM (n=340)	Controls (n=126)	χ^2 test
Nephropathy (%)	27	41	16	p<0.0001
Retinopathy (%)	31	17	0	p<0.005
Neuropathy (%)	13	19	0	p<0.005
Ischemic heart disease (%)	5	26	2	p<0.0001
Chronic limb ischemia (%)	3	11	0	p<0.05
Stroke (%)	3	8	2	ns
Insulin (%)	100	55	0	p<0.0001
OAD (%)	5	80	0	p<0.0001
Statin (%)	25	62	20	p<0.001
ACEI/ARB (%)	41	76	29	p<0.001

Regarding biochemical parameters, significant differences were observed in sRAGE concentrations of diabetic patients according to their *FN3K* (rs1056534) and *FN3K* (rs3848403) genotype (Table 4). While GG and CG genotypes of *FN3K* (rs1056534) polymorphism with mutated G allele were associated with significant decrease of sRAGE concentration (GG: 1055±458 and CG: 983±363 vs. CC: 1796±987 ng/l; p<0.0001), in *FN3K* (rs3848403) polymorphism TT genotype with mutated T allele was related with significant sRAGE increase (TT: 1365±852 vs. CT: 1016±401 and CC: 1087±508 ng/l; p=0.05).

Significant differences in markers of endothelial activation were observed in genotype subgroups of *GLO1* (rs4746) polymorphism, while that was not seen in both *FN3K* polymorphisms (Table 5).

Table 3. Genotype and allelic frequencies in patients with T1DM, T2DM and controls.

			T1DM (n=129)	T2DM (n=340)	Controls (n=126)	χ^2 test
FN3K (rs1056534)	Alleles (%)	C	36	34	40	ns
		G	64	66	60	
	Genotypes (%)	CC	15	13	20	ns
		GG	44	44	41	
		CG	41	43	39	
FN3K (rs3848403)	Alleles (%)	C	44	46	47	ns
		T	56	54	53	
	Genotypes (%)	CC	18	21	27	ns
		TT	29	30	32	
		CT	53	49	41	
GLO1 (rs4746)	Alleles (%)	C	46	49	48	ns
		A	54	51	52	
	Genotypes (%)	AA	32	28	32	ns
		CC	24	27	28	
		AC	44	45	41	

Table 4. sRAGE concentration in diabetic patients and controls in respect of their genotype.

sRAGE (ng/l)		Diabetes (n=102)	Controls (n=24)
FN3K (rs1056534)	**CC**	1796 ± 987	370 ± 135
	GG	1055 ± 458	953 ± 439
	CG	983 ± 363	811 ± 184
	ANOVA	**p<0.0001**	ns
FN3K (rs3848403)	**CC**	1087 ± 508	1265 ± 254
	TT	1365 ± 852	643 ± 128
	CT	1016 ± 401	701 ± 266
	ANOVA	**p=0.05**	ns
GLO1 (rs4746)	**AA**	1164 ± 601	853 ± 115
	CC	1035 ± 579	645 ± 302
	AC	1104 ± 449	954 ± 440
	ANOVA	ns	ns

Discussion

This is the first study describing significant relationship of *FN3K* (rs1056534) and (rs3848403) polymorphisms with concentration of sRAGE in patients with diabetes. While mutation in rs1056534 relates to lower sRAGE, mutation in rs3848403 is associated with higher sRAGE. It is still controversial, how to interpret the sRAGE level. Patients with expressed vascular damage in diabetes have higher sRAGE concentration (El-Mesallamy *et al.* 2011). Similar results were also observed in patients with decreased renal function (Kalousova *et al.* 2006, Skrha *et al.* 2012). On the other hand, sRAGE could be also a protective factor against oxidative stress and endothelial dysfunction in atherosclerosis (Santilli *et al.* 2007). There are inconsistent results regarding sRAGE as a marker of future chronic disease risk and mortality (Kalousova *et al.* 2007, Selvin *et al.* 2013). One plausible explanation suggests, that many studies did not distinguish total sRAGE vs. esRAGE components in the experiments, and therefore there are such diverse results across the studies (Daffu *et al.* 2013). In our study, the total pool of soluble RAGE with Quantikine immunoassay was measured and therefore we cannot discern whether the different variants of sRAGE (C-truncated RAGE or esRAGE) have the association to *FN3K* polymorphisms. However, strong correlation of sRAGE and esRAGE in chronic hemodialysis patients (r=0.95, P<0.001) was reported previously (Kalousova *et al.* 2007) and both sRAGE and

Table 5. Adhesion molecules concentration in patients with diabetes in respect of their *GLO1* (rs4746) genotype.

GLO1	**T1DM (n=50)**				**T2DM (n=52)**			
(rs4746)	**AA**	**CC**	**AC**	*ANOVA*	**AA**	**CC**	**AC**	*ANOVA*
ICAM (μg/l)	279 ± 65	223 ± 47	213 ± 56	**p<0.005**	281 ± 57	271 ± 102	274 ± 95	ns
VCAM (μg/l)	884 ± 308	839 ± 298	849 ± 237	ns	719 ± 314	1219 ± 426	927 ± 296	**p<0.01**
P-selectin (μg/l)	114 ± 41	78 ± 33	85 ± 39	**p<0.05**	114 ± 48	109 ± 46	110 ± 45	ns
E-selectin (μg/l)	29 ± 11	24 ± 13	32 ± 13	ns	51 ± 10	40 ± 25	34 ± 12	**p<0.05**

esRAGE increase in oxidative stress (Piarulli *et al.* 2013).

We observed significant differences in markers of endothelial activation in genotype subgroups of *GLO1* (rs4746) polymorphism in patients with diabetes. The results are though uneasy to interpret, since patients with mutated C allele had significantly reduced concentration of ICAM and P-selectin in case of T1DM, and similarly lower concentration of E-selectin in T2DM. On the other hand, significant increase in VCAM concentration was observed in the same genotypes of T2DM. To the best of our knowledge, such data have not been published yet. It is though plausible, that the concentrations of adhesion molecules are apart from *GLO1* genotype dependent on many other and dynamic factors, such as oxidative stress, stage of vascular impairment and others (Urso and Caimi 2011, Ugurlu *et al.* 2013).

The absence of significant differences in genotype and allelic frequencies of *GLO1* (rs4746) polymorphism was reported previously in patients with breast cancer (Germanova *et al.* 2009), but not in both Type 1 and Type 2 diabetes. We confirmed lack of significant differences in *FN3K* (rs1056534) and *FN3K* (rs3848403) genotype and allelic frequencies in patients with diabetes and healthy controls as reported previously (Mosca *et al.* 2011). Moreover, we did not observe differences of genotype and allelic frequencies in studied polymorphisms among patients with/without diabetic vascular complications. Similar results were presented in the past (Engelen *et al.* 2009, Mohas *et al.* 2010, Tanhäuserová *et al.* 2014). On the other hand, significantly higher prevalence of cardiovascular disease and peripheral vascular disease in CC genotype of *GLO1* (rs4746) polymorphism in hemodialysis patients was observed (Kalousova *et al.* 2008). Probably, there are also other molecular mechanisms impacting gene expression and activity of enzymes involved in deglycation systems, since diabetes vascular disease is not straightforward dependent on metabolic control (Rabbani and Thornalley 2011). Indeed, variations in copy number variants (CNV) in *GLO1* gene are associated with differences in GLO I expression and function (Williams *et al.* 2009).

There are some limitations of this study. Firstly, the total number of subjects enrolled within the study is not large enough for in-depth association analyses; moreover the number of subjects with special biochemical assessment of sRAGE and adhesion molecules was even smaller. Finally, we did not measured the activity of FN3K and GLO I, since the process described previously (Allen *et al.* 1993, Delpierre *et al.* 2006) was not easily practicable.

In conclusion, we demonstrate for the first time the association of rs1056534 and rs3848403 of fructosamine 3-kinase gene with sRAGE in patients with diabetes. In the same cohort, we found significant association of *GLO1* (rs4746) polymorphism with markers of endothelial activation, although precise explanation of such relation is uneasy to submit and larger studies will be necessary for better understanding.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

AGEs – advanced glycation end-products, FN3K – fructosamine 3-kinase, GLO I – glyoxalase I, sRAGE – soluble receptor for advanced glycation end-products, vWF – von Willebrand factor, ICAM – intercellular

adhesion molecule, VCAM – vascular cell adhesion molecule, T1DM – Type 1 diabetes, T2DM – Type 2 diabetes, FPG – fasting plasma glucose, HbA_{1c} – glycated hemoglobin, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, OAD – oral antidiabetic drugs, ACEI/ARB – angiotensin-converting enzyme inhibitors/angiotensin receptor blockers.

References

ALLEN RE, LO TWC, THORNALLEY PJ: A simplified method for the purification of human red-blood-cell glyoxalase. I. Characteristics, immunoblotting, and inhibitor studies. *J Prot Chem* **12**: 111-119, 1993.

BARUA M, JENKINS EC, CHEN WQ, KUIZON S, PULLARKAT RK, JUNAID MA: Glyoxalase I polymorphism rs2736654 causing the Ala111Glu substitution modulates enzyme activity-implications for autism. *Autism Res* **4**: 262-270, 2011.

BROWNLEE M: The pathobiology of diabetic complications – a unifying mechanism. *Diabetes* **54**: 1615-1625, 2005.

CAI WJ, RAMDAS M, ZHU L, CHEN X, STRIKER GE, VLASSARA H: Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. *Proc Nat Acad Sci USA* **109**: 15888-15893, 2012.

DAFFU G, DEL POZO CH, O'SHEA KM, ANANTHAKRISHNAN R, RAMASAMY R, SCHMIDT AM: Radical roles for RAGE in the pathogenesis of oxidative stress in cardiovascular diseases and beyond. *Int J Mol Sci* **14**: 19891-19910, 2013.

DELPIERRE G, VAN SCHAFTINGEN E: Fructosamine 3-kinase, an enzyme involved in protein deglycation. *Biochem Soc Trans* **31**: 1354-1357, 2003.

DELPIERRE G, VEIGA-DA-CUNHA M, VERTOMMEN D, BUYSSCHAERT M, VAN SCHAFTINGEN E: Variability in erythrocyte fructosamine 3-kinase activity in humans correlates with polymorphisms in the FN3K gene and impacts on haemoglobin glycation at specific sites. *Diabetes Metab* **32**: 31-39, 2006.

EL-MESALLAMY HO, HAMDY NM, EZZAT OA, REDA AM: Levels of soluble advanced glycation end product-receptors and other soluble serum markers as indicators of diabetic neuropathy in the foot. *J Invest Med* **59**: 1233-1238, 2011.

ENGELEN L, FERREIRA I, BROUWERS O, HENRY RMA, DEKKER JM, NIJPELS G, HEINE RJ, VAN GREEVENBROEK MMJ, VAN DER KALLEN CJH, BLAAK EE, FESKENS EJM, TEN CATE H, STEHOUWER CDA, SCHALKWIJK CG: Polymorphisms in glyoxalase 1 gene are not associated with vascular complications: the Hoorn and CoDAM studies. *J Hypertens* **27**: 1399-1403, 2009.

GENUTH S, SUN WJ, CLEARY P, SELL DR, DAHMS W, MALONE J, SIVITZ W, MONNIER VM, ANCILLARY DSC: Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* **54**: 3103-3111, 2005.

GERMANOVA A, TESAROVA P, JACHYMOVA M, ZVARA K, ZIMA T, KALOUSOVA M: Glyoxalase I Glu111Ala polymorphism in patients with breast cancer. *Cancer Invest* **27**: 655-660, 2009.

GIACCO F, DU X, D'AGATI VD, MILNE R, SUI G, GEOFFRION M, BROWNLEE M: Knockdown of glyoxalase 1 mimics diabetic nephropathy in nondiabetic mice. *Diabetes* **63**: 291-299, 2014.

KALOUSOVA M, HODKOVA M, KAZDEROVA M, FIALOVA J, TESAR V, DUSILOVA-SULKOVA S, ZIMA T: Soluble receptor for advanced glycation end products in patients with decreased renal function. *Am J Kidney Dis* **47**: 406-411, 2006.

KALOUSOVA M, JACHYMOVA M, MESTEK O, HODKOVA M, KAZDEROVA M, TESAR V, ZIMA T: Receptor for advanced glycation end products – soluble form and gene polymorphisms in chronic haemodialysis patients. *Nephrol Dial Transplant* **22**: 2020-2026, 2007.

KALOUSOVA M, GERMANOVA A, JACHYMOVA M, MESTEK O, TESAK V, ZIMA T: A419C (E111A) polymorphism of the glyoxalase I gene and vascular complications in chronic hemodialysis patients. *Ann NY Acad Sci* **1126**: 268-271, 2008.

KUSUNOKI H, MIYATA S, OHARA T, LIU BF, URIUHARA A, KOJIMA H, SUZUKI K, MIYAZAKI H, YAMASHITA Y, INABA K, KASUGA M: Relation between serum 3-deoxyglucosone and development of diabetic microangiopathy. *Diabetes Care* **26**: 1889-1894, 2003.

LEVEY AS, BOSCH JP, LEWIS JB, GREENE T, ROGERS N, ROTH D: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* **130**: 461-470, 1999.

LINDSEY JB, CIPOLLONE F, ABDULLAH SM, MCGUIRE DK: Receptor for advanced glycation end-products (RAGE) and soluble RAGE (sRAGE): cardiovascular implications. *Diabetes Vasc Dis Res* **6**: 7-14, 2009.

MIYATA T, DE STRIHOU CV, IMASAWA T, YOSHINO A, UEDA Y, OGURA H, KOMINAMI K, ONOGI H, INAGI R, NANGAKU M, KUROKAWA K: Glyoxalase I deficiency is associated with an unusual level of advanced glycation end products in a hemodialysis patient. *Kidney Int* **60**: 2351-2359, 2001.

MOHAS M, KISFALI P, BARICZA E, MEREI A, MAASZ A, CSEH J, MIKOLAS E, SZIJARTO 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 Diab* **118**: 209-212, 2010.

MOSCA L, PENCO S, PATROSSO MC, MAROCCHI A, LAPOLLA A, SARTORE G, CHILELLI NC, PALEARI R, MOSCA A: Genetic variability of the fructosamine 3-kinase gene in diabetic patients. *Clin Chem Lab Med* **49**: 803-808, 2011.

PIARULLI F, LAPOLLA A, RAGAZZI E, SUSANA A, SECHI A, NOLLINO L, COSMA C, FEDELE D, SARTORE G: Role of endogenous secretory RAGE (esRAGE) in defending against plaque formation induced by oxidative stress in type 2 diabetic patients. *Atherosclerosis* **226**: 252-257, 2013.

POPOVA EA, MIRONOVA RS, ODJAKOVA MK: Non-enzymatic glycosylation and deglycating enzymes. *Biotechnol Biotec Eq* **24**: 1928-1935, 2010.

RABBANI N, THORNALLEY PJ: Glyoxalase in diabetes, obesity and related disorders. *Semin Cell Dev Biol* **22**: 309-317, 2011.

ROBERTS AC, PORTER KE: Cellular and molecular mechanisms of endothelial dysfunction in diabetes. *Diab Vasc Dis Res* **10**: 472-482, 2013.

SANTILLI F, BUCCIARELLI L, NOTO D, CEFALU AB, DAVI V, FERRANTE E, PETTINELLA C, AVERNA MR, CIABATTONI G, DAVI G: Decreased plasma soluble RAGE in patients with hypercholesterolemia: effects of statins. *Free Radic Biol Med* **43**: 1255-1262, 2007.

SELVIN E, HALUSHKA MK, RAWLINGS AM, HOOGEVEEN RC, BALLANTYNE CM, CORESH J, ASTOR BC: sRAGE and risk of diabetes, cardiovascular disease, and death. *Diabetes* **62**: 2116-2121, 2013.

SILVA MS, GOMES RA, FERREIRA AEN, FREIRE AP, CORDEIRO C: The glyoxalase pathway: the first hundred years ... and beyond. *Biochem J* **453**: 1-15, 2013.

SKRHA J JR, KALOUSOVA M, SVARCOVA J, MURAVSKA A, KVASNICKA J, LANDOVA L, ZIMA T, SKRHA J: Relationship of soluble RAGE and RAGE ligands HMGB1 and EN-RAGE to endothelial dysfunction in type 1 and type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes* **120**: 277-281, 2012.

SONG F, SCHMIDT AM: Glycation and insulin resistance: novel mechanisms and unique targets? *Arterioscler Thromb Vasc Biol* **32**: 1760-1765, 2012.

TANHÄUSEROVÁ V, KURICOVÁ K, PÁCAL L, BARTÁKOVÁ V, ŘEHOŘOVÁ J, SVOJANOVSKÝ J, OLŠOVSKÝ J, BĚLOBRÁDKOVÁ J, KAŇKOVÁ K: Genetic variability in enzymes of metabolic pathways conferring protection against non-enzymatic glycation versus diabetes-related morbidity and mortality. *Clin Chem Lab Med* **52**: 77-83, 2014.

UGURLU N, GERCEKER S, YULEK F, UGURLU B, SARI C, BARAN P, CAGIL N: The levels of the circulating cellular adhesion molecules ICAM-1, VCAM-1 and endothelin-1 and the flow-mediated vasodilatation values in patients with type 1 diabetes mellitus with early-stage diabetic retinopathy. *Intern Med* **52**: 2173-2178, 2013.

URSO C, CAIMI G: Oxidative stress and endothelial dysfunction. *Minerva Medica* **102**: 59-77, 2011.

VLASSARA H, STRIKER GE: Advanced glycation endproducts in diabetes and diabetic complications. *Endocrin Metab Clin* **42**: 697-719, 2013.

WILLIAMS R, LIM JE, HARR B, WANG C, WALTERS R, DISTLER MG, TESCHKE M, WU CL, WILTSHIRE T, SU AI, SOKOLOFF G, TARANTINO LM, BOREVITZ JO, PALMER AA: A common and unstable copy number variant is associated with differences in Glo1 expression and anxiety-like behavior. *Plos One* **4**: article number e4649, 2009.

YAN SF, D'AGATI V, SCHMIDT AM, RAMASAMY R: Receptor for Advanced Glycation Endproducts (RAGE): a formidable force in the pathogenesis of the cardiovascular complications of diabetes & aging. *Curr Mol Med* **7**: 699-710, 2007.

YONEKURA H, YAMAMOTO Y, SAKURAI S, PETROVA RG, ABEDIN MJ, LI H, YASUI K, TAKEUCHI M, MAKITA Z, TAKASAWA S, OKAMOTO H, WATANABE T, YAMAMOTO H: Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J* **370**: 1097-1109, 2003.

ZIEMAN SJ, MELENOVSKY V, CLATTENBURG L, CORRETTI MC, CAPRIOTTI A, GERSTENBLITH G, KASS DA: Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. *J Hypertens* **25**: 577-583, 2007.