Association of the Receptor for Advanced Glycation End Products (RAGE) -374 T/A Gene Polymorphism and Circulating Soluble RAGE with Nephropathy in Type 1 Diabetic Patients

[1]Hala A. Abdel-Azeez and [2]Amir M. El-Okely

[1]Clinical Pathology and [2]Internal Medicine Departments, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

The binding of advanced glycation end-products (AGEs) to their receptor (RAGE) may play an important role in the development of diabetic vascular complications. Circulating soluble RAGE (sRAGE) reflects tissue RAGE expression. We examined circulating sRAGE and RAGE -374 T/A gene promoter polymorphism in type 1 diabetic patients and explored their possible associations with the development of nephropathy. Fifty diabetic patients with disease duration > 10 years and 20 age, sex and body mass index (BMI) matched healthy controls were included in the study. Diabetic patients were subdivided into 23 patients without nephropathy and 27 with nephropathy. All the studied individuals were subjected to the following investigations: fasting glucose, HbA_{1c}, serum creatinine, lipid profile, albuminuria and sRAGE levels. The -374 T/A RAGE gene polymorphism was studied by PCR amplification and restriction fragment length polymorphism (RFLP) analysis. Our study reported significant increase in sRAGE in diabetic patients compared to controls and in diabetic patients with nephropathy compared to those without nephropathy (*P*<0.001). sRAGE was significantly correlated with HbA_{1c}, creatinine, albuminuria and atherogenic lipid profile. There were significant increase in the frequency of RAGE -374 A allele (T/A and/or A/A genotypes) in diabetic patients with nephropathy compared to those without nephropathy and control groups (P<0.01). A allele was a risk factor for diabetic nephropathy (OR 2.36 &95%CI 1.1-5.6). RAGE -374 A allele was associated with increased sRAGE levels, hypertension and increased creatinine concentration in diabetic patients. This study points to the possible role of sRAGE as a marker of early nephropathy in diabetic patients. Early testing for the RAGE gene -374 T/A could have merit in predicting risk of diabetic nephropathy later in life.

Diabetic nephropathy is a serious long-term consequence of diabetes, affecting both type 1 and type 2 diabetic patients. It has been assumed that the underlying pathophysiological mechanism leading to late diabetic complications are similar in type 1 and type 2 diabetes, but different modifying factors may be operative in the two forms of diabetes, including genetic factors. Type 2 diabetes mellitus is the most common form of diabetes that constitute the majority of cases worldwide including Egypt (Abou-Shousha *et al.* ,2006) and the incidence of nephropathy is higher in type 2 diabetes compared to type 1 (Yokoyama *et al.,*2000). However, increased risk of microangiopathy was much more complex in type 2 diabetes, suggesting that other factors, such as insulin resistance, hypertension, obesity, dyslipidaemia and ischemic renal disease, might be of greater importance in the development of diabetic complications in type 2 than in type 1 diabetes and the contribution of the genetic factors may be obscure as a results (Lindholm *et al.,* 2006).

In individuals with diabetes, nonenzymatic glycation of proteins leads to the formation of advanced glycation end products (AGE) and this process occurs at an accelerated rate in chronic hyperglycemia. Increasing evidence suggests that AGE contribute to the development of diabetic vascular complications (Wendt *et al*., 2003a). The effects of AGE are partially mediated through their interactions with cell surface receptors. Among these receptors, RAGE (receptor for

the AGE) is the best characterized (Bucala & Vlassara, 1995). It is now well established that ligand-triggered RAGE-dependent cellular activation induces inflammatory response and promotes cellular migration and proliferation (Schmidt *et al*., 1999). The binding of AGE to RAGE elicits oxidative stress generation and subsequently evokes vascular injury in diabetes (Ueda *et al*., 2006). In addition, pharmacological blockade of the RAGE receptor prevents untoward effects of AGE on the vascular wall in diabetic animals (Bucciarelli *et al*., 2002a).

RAGE is a multiligand receptor of the immunoglobulin superfamily expressed at low levels in adult tissues in homeostasis, but highly overexpressed at sites of vascular pathology (Bucciarelli *et al*., 2002b). Isoforms of the RAGE derived from alternative splicing of the RAGE gene mRNA have been found. The full-length protein is the fully functional form (Yan *et al.,* 2003). Two other isoforms have been detected: an N-truncated form that is located mainly on the plasma membrane and is incapable of binding AGEs, and a C-truncated form that lacks the transmembrane domain. The C-truncated isoform is a soluble RAGE protein (sRAGE) secreted from cells and its ability to bind RAGE ligands suggests a cytoprotective action against AGEs (Yonekuura *et al.,* 2003).

As diabetics differ markedly in their susceptibility to glucose–mediated tissue damage due to non enzymatic glycation, RAGE is regarded as one of the logical candidate genes involved in the familial predisposition to certain types of diabetic long term complications (Kankova *et al.,* 2001). Sequence variants within the RAGE gene may influence development of complications by altering the AGE-RAGE interaction (Hudson *et al.,* 1998). Several polymorphisms of the RAGE gene have been described (Hudson *et al.,* 2002). Specifically three polymorphisms of the promoter region, –429T/C, –374T/A and a 63-bp deletion, were shown to be functional, increasing gene transcription. The introduction of a T→A nucleotide substitution in the –374 T/A polymorphism apparently prevents the binding of a nuclear binding factor that is probably involved in the repression of RAGE transcription; thus, the presence of the –374 A allele increases the promoter transcription activity approximately three-fold in vitro (Hudson *et al.,* 2001a). The -374 T/A variant has thus become a topic of great interest. The association of this promoter polymorphism has been studied in various populations with respect to diabetic complications, but the results are conflicting (Pettersson –Fernholm *et al.,* 2003; Ji Xiong *et al.,* 2003; Hudson *et al.,* 2001a; Kirbis *et al.,* 2004; Kankova *et al.,* 2005; dos Santos *et al.,* 2005).

The aim of the study was to examine circulating sRAGE and RAGE -374 T/A gene polymorphism in type 1 diabetic patients and evaluate their possible associations with the development of nephropathy.

Subjects and Methods

A total of 50 type 1 diabetic patients with disease duration >10 years attending Diabetes Clinic of Zagazig University Hospitals and 20 healthy age-, sex-, and BMI-matched controls were enrolled in this study. Diagnosis of diabetes was done according to the American Diabetes Association Criteria (2006). Patients with marked renal dysfunction (serum creatinine > 2.0 mg/dl) and patients treated with angiotensin converting enzyme inhibitors or angiotensin- receptor blockers were excluded from the study as these factors were reported to modify sRAGE blood level (Grossin *et al.,* 2008; Kalousova *et al.,* 2007). Informed consent was obtained from all participants in the study.

Urine albumin excretion (UAE) rates were measured from 24 hours urine collections on BN Prospec (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). According to UAE, diabetic patients were subdivided into 23 patients without nephropathy (normoalbumiuric) and 27 with nephropathy (micro- or macroalbumiuric). Normo-, micro-, and macroalbumiuria were defined as UAE

<30, 30-300, and >300 mg/24 h. respectively (Sacks, 2006). The two patients groups are matched for disease duration.

Hypertension was defined by a systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg and/or by taking anti-hypertensive agents (Chobanian *et al.,* 2003). Presence of ischemic heart disease (IHD) was recorded by previous history of angina or myocardial infarction. The presence of diabetic retinopathy was confirmed by ophthalmologists. Patients' clinical characteristics are summarized in table 1.

Blood samples were taken from all the studied subjects. Aliquots of sera were stored at -20˚C for sRAGE determination. Measurement of serum glucose, creatinine, total cholesterol, triglycerides were done on ADVIA 1650 analyzer (Siemens). High density lipoprotein cholesterol (HDL-C) was determined using Elitech kit (Elitech Diagnostics, Sees, France) (Burstein *et al.,* 1970). Low density lipoprotein-cholesterol (LDL-C) was calculated according to Friedewald's equation (Friedewald *et al.,* 1972). Patients were considered dyslipidemic if they were taking lipid lowering agents and/or if serum cholesterol was >200 mg/dl, LDL-cholesterol was > 130 mg/dl, and HDL-cholesterol was < 40 mg/dl according to the criteria of National Cholesterol Education Program Expert Panel (2001). Glycated hemoglobin (HbA_{1c}) was determined on EDTA blood using Biosystem kit (Barchelona, Spain).

sRAGE were measured by sandwich ELISA technique using commercially available kit (Quantikine; R&D system, Minneapolis, MN, USA) according to the manufacturer's protocol. Intra-assay and inter-assay precision CV (%) ranges are 4.8-6.1 and 6.7-8.2 respectively. The minimal detection concentration was 4.12 pg/ml.

Table 1. Patients' Clinical Characteristics.

Variables	Diabetic Patients		*P* value
	Without Nephropathy (n=23)	With Nephropathy (n=27)	
Age (years)	45.9±6.3 (35-58)	46.96±6.4 (37-59)	NS
Sex (M/F)[a]	13/10(56.5/43.5)	15/12(55.6/44.4)	NS
BMI (kg/m^2)	26.2±3.1 (20.0-31.4)	25.8±4.6 (19.0-37.1)	NS
Diabetes duration (years)	15.6±1. 8 (13.1-19.4)	15.9±2. 0 (13.5-21.0)	NS
Hypertension[a]	8(34.8)	16(59.3)	NS
Retinopathy[a]	5(21.7)	13(48.1)	NS
IHD[a]	2(8.7)	7(25.9)	NS
Neuropathy[a]	3(13.0)	5 (18.5)	NS
Micro/macroalbuminuria	-	9/18(33.3/66.7)	

n= the numbers of subjects. Values are represented as mean ±SD (range), or n (%)[a].
P>0.05 is not significant. NS= not significant

-374 RAGE Genotyping

Total genomic DNA was purified from buffy coat samples of EDTA blood using the E. Z. N. A. ™ Blood DNA Kit (Omega – biotek. Inc) as recommended by the manufacturer. Genomic DNA samples were stored at -20ºC until genotyping analysis. The A/T transversion polymorphism at position -374 in the promotor region of the RAGE gene was analyzed by PCR-restriction fragment length polymorphism (RFLP) method as previously described (Falcone *et al,* 2004). A 671-bp PCR fragment of the RAGE promoter region was amplified using the following primers: forward, 5'-CCTGGGTTTAGTTGAGATTTTTT-3' and reverse, 5'- GAAGGCACTTCCTCGGGTTCT-3'. PCR amplification of DNA was performed by puReTaq Ready- To- Go PCR Beads (Amersham Biosciences). Reactions were performed in a total volume of 25 µl containing 100 ng of template DNA, 25 pM of each primer (Operon Biotechnologies) in a tube containing one PCR bead [200 µM of each dNTP in 10 mM Tris - HCL (pH 9.0), 50 mM HCL and 1.5 mM MgCl2 and 2.5 U Taq DNA polymerase]. The following temperature scheme was performed for the amplification using thermal cycler (Gene Amp, PCR system 9700) : a denaturing step of 94°C for 2 min, followed by 30 cycles of 94°C for 30 sec, 58°C for 30 sec, 72°C for 1 min, and a final incubation at 72°C for

10 min. After amplification, the products were digested with *Tsp509 I* (New England Biolabs, Cambridge, MA, USA) at 65°C for 16 h, and were run on an ethidium-bromide stained 3% agarose gel. *Tsp509 I* digestion of the 671-bp fragments were cut into five fragments of 284, 217, 110, 44 and 16 bp (allele T) or four fragments of 327, 284, 44 and 16 bp (allele A) (figure1).

Statistical Analysis

Data were expressed as mean ± SD for quantitative variables, number and percentage for qualitative ones. ANOVA, t test, χ^2, and Pearson correlation were used for analysis of results. Odds ratio (OR) and 95% confidence interval (CI) were performed to predict the effect of RAGE genotypes on development of diabetes and diabetic nephropathy. A *P*-value < 0.05 was considered significant. All statistical calculations were done using SPSS computer program (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for the Microsoft Windows.

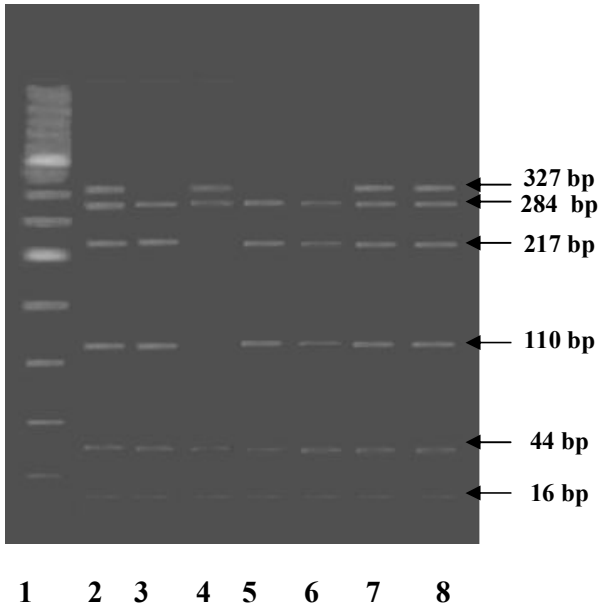

Figure 1. Electrophoresis on 3% Agarose Gel of The -374 T/A RAGE Gene Polymorphism PCR Products. Lane 1:50 bp DNA Ladder (Norgen Biotek), Lanes 3, 5, 6: TT Genotype, Lane 4: AA Genotype, and Lanes 2, 7, 8: TA Genotype.

Results

The laboratory investigations of the studied groups are presented in table 2. Diabetic groups had significantly higher fasting glucose, HbA_{1c}, lower HDL-C than control group. Patients with diabetic nephropathy had significantly increased serum creatinine, total cholesterol, triglycerides, and LDL-C compared to those without nephropathy. sRAGE was significantly increased in diabetic patients without nephropathy compared to control groups, and in diabetic patients with nephropathy compared to those without nephropathy and control groups (P<0.001).

On studying the correlation between sRAGE level and other studied laboratory parameters in diabetic patients, sRAGE was significantly correlated with HbA_{1c} (P<0.01), serum creatinine (P<0.001), total cholesterol (P<0.001), triglycerides (<0.01), LDL-C (P<0.001), and UAE rate (P<0.001). Fasting glucose, HDL-C were not significantly correlated with sRAGE (P>0.05) (table 3).

Table 2. Laboratory Investigations of the Studied Groups.

Variables	Control group (n=20)	Diabetic patients		*P* value
		Without nephropathy (n=23)	With nephropathy (n=27)	
Glucose(mg/dl)	87.6±10.8* (70-100)	169.5±42.5 (98-281)	160.0±41.2 (102-256)	<0.001
HbA_{1c} (%)	4.9±0.54* (4.1-5.9)	7.5±0.8 (6.2-9.0)	7.7±0.64 (6.4-9.0)	<0.001
Creatinine(mg/dl)	0.8±0.195 (0.5-1.4)	1.0±0.24* (0.6-1.4)	1.5±0.35* (0.9-2.0)	<0.001
Total cholesterol(mg/dl)	183.8±22.4 (151-236)	198.9±23.5 (159-251)	221.2±38.96* (161-298)	<0.001
Triglycerides(mg/dl)	115.95±20.5 (74-150)	153.1±35.9* (96-211)	183.0±43.1* (111-280)	<0.001
HDL-C (mg/dl)	51.4±7.1* (42-67)	44.3±4.5 (35-51)	46.1±5.5 (35-55)	<0.001
LDL-C (mg/dl)	108.7±15.7 (85-130)	124.5±20.98* (89-174)	138.7±32.3* (80-195)	<0.01
sRAGE (pg/ml)	911.3±272.5 (489-1303)	1210.4±432.0* (616-1950)	1452.0±475.2* (751-2450)	<0.001

n= the numbers of subjects.

Values are represented as mean± SD (range). *P*<0.05 is significant.

*Significantly different from other groups.

Table 3. Correlation Between sRAGE Levels (pg/ml) and Other Studied Laboratory Variables in Diabetic Patients.

Variables	r	*P* value
Glucose (mg/dl)	0.09	NS
HbA_{1c} (%)	0.38	<0.01
Creatinine (mg/dl)	0.72	<0.001
Total cholesterol (mg/dl)	0.57	<0.001
Triglycerides (mg/dl)	0.4	<0.01
HDL-C (mg/dl)	0.12	NS
LDL-C (mg/dl)	0.57	<0.001
UAE rates (mg/24h)	0.69	<0.001

P<0.05 is significant. NS= not significant

Table 4 summarizes genotypes distribution and alleles frequencies of RAGE -374 T/A gene in the studied groups. Due to few numbers of cases with A/A genotype, T/A and A/A genotypes were considered as one group. A/A genotype was present in 4 patients with nephropathy, while it was absent in the other two groups. There were significant increase in T/A and A/A genotype and decrease in T/T genotype in diabetic patients with nephropathy compared to patients without nephropathy and controls. Patients with

nephropathy had significantly higher frequency of RAGE -374 A allele compared to patients without nephropathy and control groups. Genotype distribution and allele frequency were not significantly different between diabetic patients without nephropathy and control groups.

There was no significant association between RAGE -374 T/A gene polymorphism and the risk of diabetes. The odds ratio of development of diabetes in individuals carrying RAGE -374 A allele was 2.2(95% CI 0.69-6.99). There was significant increased risk of development of nephropathy in diabetic patients (OR 2.36 & 95% CI 1.1-5.6).

The association between carrying RAGE -374 A allele and other variables in diabetic patients are reported in table 5. Hypertension and increased creatinine concentration were associated with carrying RAGE -374 A allele in diabetic patients (P<0.05, P<0.001 respectively). sRAGE was significantly increased in diabetic patients carrying A allele compared to non A carriers(P<0.001). While no significant association was found between carrying A allele and other variables in diabetic patients (P>0.05).

Table 4. -374 T/A RAGE Genotype Distribution and Allele Frequencies in The Studied Groups.

Variables	Control group (n=20)	Diabetic patients		*P* value
		Without nephropathy (n=23)	With nephropathy (n=27)	
Genotypes [a]				
TT	15 (75)	12 (52.2)	8 (33.3)*	
TA/AA	5 (25)	11 (47.8)	19 (66.7) *	<0. 01
Allele frequencies				
T allele	0.88	0.67	0.57*	<0.01
A allele	0.12	0.33	0.43*	

n= the numbers of subjects. [a]Results are represented as n (%).

*Significantly different from other groups. P<0.05 is significant.

Table 5. Association Between Carrying RAGE -374 A Allele and Other Variables in Diabetic Patients.

Variable	Non A- carriers (n=20)	A- carriers (n=30)	*P* value
Hypertension[a]	13 (65)	11 (36.7)	<0.05
Retinopathy[a]	6 (30)	12 (40)	NS
Neuropathy[a]	5(25)	3(10)	NS
IHD[a]	4(20)	3(10)	NS
Dyslipidemia[a]	4(20)	2(6.7)	NS
HbA_{1c} (%)	7.4±0.7 (6.4-8.9)	7.7±0.7 (6.2-9.0)	NS
Albuminuria[a]	8(40)	19(63.3)	NS
Creatinine (mg/dl)	1.0±0.24 (0.6-1.4)	1.4±0.38 (0.7-2.0)	<0.001
sRAGE (pg/ml)	982.7±297.8 (616-1831)	1579.7±404.2 (981-2450)	<0.001

n=the numbers of subjects. Values are represented as mean± SD (range), or n(%)[a].

P<0.05 is significant. NS= not significant

Discussion

Several studies have provided evidence that RAGE may play a critical role in diabetic vascular complications. Our study reported significant increased sRAGE levels in diabetic groups compared to controls and in diabetic patients with nephropathy compared to those without nephropathy. These results are in accordance with previous studies demonstrating increased sRAGE levels in type 1 diabetes (Forbes *et al.,* 2005; Challier *et al.,* 2005) and type 2 diabetes (Nakamura *et al.,* 2007b; Humpert *et al.,* 2007; Nakamura *et al.,* 2008;Gohda *et al.,* 2008). The positive association of sRAGE with nephropathy was also previously reported (Gohda *et al.,* 2008; Humpert *et al.,* 2006; Humpert *et al.,* 2007). We reported significant positive correlations of sRAGE levels with HbA_{1c}, creatinine, UAE rate and atherogenic lipid profile in diabetic patients. Other studies reported positive correlation of sRAGE with albuminuria (Humpert *et al.,* 2006; Humpert *et al.,* 2007), and serum creatinine (Tan *et al.,* 2006; Nakamura *et al.,* 2007b). Hence, serum sRAGE levels might represent an early marker of microvascular dysfunction and diabetic nephropathy.

In contrast, other studies reported decreased level of sRAGE in diabetic patients and its vascular complications. Plasma sRAGE consists of an endogenous splice variant of RAGE lacking the transmembrane domain of the receptor (esRAGE) (Yonekura *et al.,* 2003) as well as proteolytically cleaved forms shed into the bloodstream by action of extracellular metalloproteinases (Hudson *et al.,* 2005). The reasons for the contradictory findings reported above may be explained by the fact that different variants of sRAGE are detected by different assays used in different studies, namely the total pool of sRAGE or specifically esRAGE. It seems likely that sRAGE and esRAGE are distinct markers, may have different functions and therefore they are not inter-exchangeable (Nakamura *et al.,* 2007b). We measured total sRAGE which is approximately four to fivefold higher than esRAGE (Katakami *et al.,* 2005).

This naturally occurring form of sRAGE, as well as artificially produced sRAGE, can potentially bind to an AGE ligand thereby acting as a decoy, preventing AGE–RAGE interaction and activation (Yonekura *et al.,* 2003). Although sRAGE has been an interesting subject of investigation under this suspected decoy function, it is very unlikely that sRAGE can act as such (i. e. by capturing and eliminating AGEs) because the sRAGE concentrations found in plasma are approximately 1,000 times lower than needed for efficient binding and elimination of AGEs (Humpert *et al.,* 2007; Nakamura *et al.,* 2008). The increased levels of sRAGE in individuals with diabetic nephropathy seem not to sustain such a decoy function.

Family clustering, apparent heterogeneity in the onset and progression of diabetic nephropathy in diabetes and the results of segregation studies indicate that genetic factors contribute to susceptibility to diabetic nephropathy (kankova *et al.,* 2005). The AGE–RAGE pathway has been extensively studied in the context of diabetic vascular disease. Of the many RAGE gene polymorphisms that have been screened, -374 T/A is considered important, since this polymorphism is reported to increase the RAGE gene transcript, which will augment increased AGE binding to cells and as a result lead to an altered signaling cascade (Hudson *et al.,* 2001a). Our study reported significant increase in the frequency of RAGE -374 A allele (T/A and/or A/A genotypes) in diabetic patients with nephropathy compared to those without nephropathy and control groups. The

odds ratio of development of nephropathy in diabetic patients was 2.36.

Association results from studies performed on the -374 T/A RAGE polymorphism have not been consistent between different populations. Lindholm *et al. (*2006) reported that the minor allele A was more frequent in type 1 diabetic patients than in non-diabetic control subjects and is a risk factor for diabetic nephropathy and sight threatening retinopathy in type 1 diabetic subjects. Ramprasad *et al.* (2007) observed association of the -374 A allele with the nonproliferative diabetic retinopathy, while the allele frequencies between the nondiabetic and diabetic groups were similar. Several other studies support the role of A allele as a risk factor against diabetic complications (Hudson *et al.,* 2001a; Yamamoto *et al.,* 2001; Wendt *et al.,* 2003b). However, other studies have failed to demonstrate an association between this polymorphism and diabetic vascular complications (Ji Xiong *et al.,* 2003; Hudson *et al.,* 2001b).

Our findings that the A allele was significantly associated with diabetic nephropathy conflicts with a study in Finnish type 1 diabetic patients, in whom the A/A genotype was protective in patients with high HbA_{1c} (Pettersson-Fernholm *et al.,* 2003). Similarly, studies on macrovascular disease in type 2 diabetic and in non-diabetic patients have shown a protective role of the A allele (Picheth *et al.,* 2007; Falcone *et al.,* 2004; Falcone *et al.,* 2005; dos Santos *et al.,* 2005). Differences in studies design and ethnic background may explain the differences observed in different studies. However, the A allele has shown to have a threefold increased transcriptional activity and it has been shown that overexpression of RAGE in a double transgenic diabetic mouse led to enhanced albuminuria, mesangial expansion and glomerulosclerosis (Yamamoto *et al.,* 2001). It would therefore be plausible to expect that the A allele is more frequent in diabetic patients with complication than without complications.

Our study revealed significant increase of sRAGE in diabetic patients carrying A allele compared to non A carriers. Hudson *et al.* (2001a) showed that –374 A mutation in the RAGE gene promoter led to an increase in its transcription in vitro, suggesting that carriers of the homozygous A/A genotype have increased RAGE expression. Nakamura *et al.* (2007a) suggested that circulating endogenous sRAGE reflects tissue RAGE expression. Thus, an increase in RAGE transcription due to the homozygous –374 A allele would considerably increase serum levels of sRAGE.

Our study reported that hypertension and increased creatinine concentration were associated with A allele carrying in diabetic patients. Lindholm *et al.* (2006) reported the association with diabetic nephropathy was independent of metabolic control, blood pressure and other known risk factors. Proteinuria and decline of renal function are probably net results of a series of events implicated in the pathogenesis of diabetic nephropathy rather than relevant intermediate phenotype for the gene studied (Kankova *et al.,* 2005).

Diabetic nephropathy is characterized by abnormal deposits of matrix material in the glomerular mesangium, leading to glomerulosclerosis (Wautier & Guillausseau, 2001). It has been shown that the activation of the AGE–RAGE axis could lead to glomerulosclerosis and glomerular hyperpermeability through the upregulation of vascular endothelial growth factor and/or transforming growth factor-β (Wendt *et al.,* 2003b). Other studies have found positive associations between sRAGE and markers of endothelial dysfunction and low grade inflammation (Nakamura *et al.,* 2007a; Nakamura *et al.,* 2008). The current view is that the activation of RAGE through ligand

binding results in activation and translocation of nuclear transcription factors and transcription of the target genes, including those for VCAM-1 (Harja *et al.,* 2008), E-selectin (Basta *et al.,* 2002) and proinflammatory cytokines (Hofmann *et al.,* 1999). Interestingly, soluble RAGE itself might be capable of triggering inflammatory reactions via binding of Mac-1 and subsequent activation of NF-κB and thus contribute to the development of vascular complications (Pullerits *et al.,* 2006).

The gene encoding RAGE is located on chromosome 6 in the major histocompatibility complex, a region of the genome containing a number of inflammatory genes (Hudson and Schmidt, 2004). Because of the strong linkage disequilibrium between different HLA loci, it is possible that at least some of the associations found with the RAGE and diabetic complications may in fact be due to variations in other genes in the same region. A few but not all previous studies have shown an association between diabetic nephropathy or retinopathy and HLA genotype (Wong *et al.,* 2002; Agardh *et al.,* 2004).

In conclusion, this study points to the possible role of sRAGE as a marker of early nephropathy in diabetic patients. Early testing for the -374 T/A allele may have merit in predicting risk of diabetic nephropathy later in life and available preventive measures could be most effectively and consistently targeted. Further studies on large number of subjects and other RAGE gene polymorphisms needed to be studied in this population to understand their impact on the onset and progression of diabetic nephropathy.

References

1. Abou-Shousha S, Abd El-Megeed MH, Sultan HK (2006). Interleukin-8, ferritin and soluble transferrin receptors in type 2 diabetes mellitus. Egypt J Immunol; 13(1):19-25.
2. Agardh E, Gaur LK, Lernmark A, Agardh CD (2004). HLADRB1, -DQA1, and -DQB1 subtypes or ACE gene polymorphisms do not seem to be risk markers for severe retinopathy in younger type 1 diabetic patients. J Diabet Comp; 18:32–6.
3. American Diabetes Association. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2006). Diabetes Care; 29:S43–8.
4. Basta G, Lazzerini G, Massaro M, Simoncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, De Caterina R (2002). Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: a mechanism for amplification of inflammatory responses. Circulation; 105:816–22.
5. Bucala R, Vlassara H (1995). Advanced glycosylation end products in diabetic renal and vascular disease. Am J Kidney Dis; 26: 875-88.
6. Bucciarelli LG, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, Goova MT, Moser B, Kislinger T, Lee DC, Kashyap Y, Stern DM, Schmidt AM (2002a). RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. Circulation; 106: 2827-35.
7. Bucciarelli LG, Wendt T, Rong L, Lalla E, Hofmann MA, Goova MT, Taguchi A, Yan SF, Yan SD, Stern DM, Schmidt AM (2002b). RAGE is a multiligand receptor of the immunoglobulin superfamily: implications for homeostasis and chronic disease. Cell Mol Life Sci; 59: 1117-28.
8. Burstein M, Scholnick HR, Morfin R (1970). Rapid method for isolation of lipoproteins from human serum by precipitation with polyanion. J Lipid Res; 11: 583-95.
9. Challier M, Jacqueminet S, Benabdesselam O, Grimaldi A, Beaudeux JL (2005). Increased serum concentrations of soluble receptor for advanced glycation endproducts in patients with type 1 diabetes. Clin Chem; 51:1749–50.
10. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Jr JLI, Jones DW, Materson BJ, Oparil S, Jr JTW, Roccella EJ (2003). The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report**.** JAMA.; 289:2560-71.
11. dos Santos KG, Canani LH, Gross JL, Tschiedel B, Pires Souto KE, Roisenberg I (2005). The −374A allele of the receptor for advanced glycation end

products gene is associated with a decreased risk of ischemic heart disease in African-Brazilians with type 2 diabetes. Mol Genet Metab; 85:149–56.

12. Falcone C, Campo I, Emanuele E, Buzzi MP, Zorzetto M, Sbarsi I, Cuccia MA (2004). Relationship between the −374T/A RAGE gene polymorphism and angiographic coronary artery disease. Int J Mol Med; 14:1061–4.
13. Falcone C, Campo I, Emanuele E, Buzzi MP, Geroldi D, Belvito C, Zorzetto M, Sbarsi L, Cuccia M (2005). −374T/A polymorphism of the RAGE gene promoter in relation to severity of coronary atherosclerosis. Clin Chim Acta; 354:111–6.
14. Forbes JM, Thorpe SR, Thallas-Bonke V, Pete J, Thomas MC, Deemer ER, Bassal S, El-Osta A, Long DM, Panagiotopoulos S, Jerums G, Osicka TM, Cooper ME (2005). Modulation of soluble receptor for advanced glycation end products by angiotensin-converting enzyme-1 inhibition in diabetic nephropathy. J Am Soc Nephrol, 16(8):2363-72.
15. Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem; 18: 499-502.
16. Gohda T, Tanimoto M, Moon J-Y, Gotoh H, Aoki T, Matsumoto M, Shibata T, Ohsawa I, Funabiki K, Tomino Y (2008). Increased serum endogenous secretory receptor for advanced glycation end-product (esRAGE) levels in type 2 diabetic patients with decreased renal function. Diabetes Res Clin Pract; 81:196–201.
17. Grossin N, Wautier M-P, Meas T, Guillausseau P-G, Massin P, Wautier J-L (2008). Severity of diabetic microvascular complications is associated with a low soluble RAGE level. Diabetes Metab; 34: 392–5.
18. Harja E, Bu DX, Hudson BI, Chang JS, Shen X, Hallam K, Kalea AZ, Lu Y, Rosario RH, Oruganti S, Nikolla Z, Belov D, Lalla E, Ramasamy R, Yan SF, Schmidt AM (2008). Vascular and inflammatory stresses mediate atherosclerosis via RAGE and its ligands in apoE−/− mice. J Clin Invest; 118:183–94.
19. Hofmann MA, Drury S, Fu C, Qu W, Taguch A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P, Neurath MF, Slattery T, Beach D, McClary J, Nagashima M, Morser J, Stern D, Schmidt AM (1999). RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/ calgranulin polypeptides. Cell; 97:889–901.
20. Hudson BI, Schmidt AM (2004). RAGE: a novel target for drug intervention in diabetic vascular disease. Pharm Res; 21:1079- 86.
21. Hudson BI, Stickland MH, Grant PJ (1998). Identification of polymorphisms in the receptor for advanced glycation end products (RAGE) gene prevalence in type 2 diabetes and ethnic groups. Diabetes; 47:1155-7.
22. Hudson BI, Stickland MH, Futers S, Grant PJ (2001a). Effects of novel polymorphisms in the RAGE gene on transcriptional regulation and their association with diabetic retinopathy. Diabetes; 50:1505–11.
23. Hudson BI, Stickland MH, Futers TS, Grant PJ (2001b). Study of the –429 T/C and –374 T/A receptor for advanced glycation end products promoter polymorphisms in diabetic and nondiabetic subjects with macrovascular disease. Diabetes Care; 24:2004.
24. Hudson BI, Hofmann MA, Bucciarelli L, Wendt T, Moser B, Lu Y, Qu W, Stern DM, D'Agati V, Yan SD, Yan SF, Grant PJ, Schmidt AN (2002). Glycation and diabetes: the RAGE connection. Curr Sci; 83:1515–20.
25. Hudson BI, Harja E, Moser B, Schmidt AM (2005). Soluble levels of receptor for advanced glycation endproducts (sRAGE) and coronary artery disease: the next C-reactive protein? Arterioscler Thromb Vasc Biol; 25(5):879-82.
26. Humpert PM, Kopf S, Djuric Z, Wendt T, Morcos M, Nawroth PP, Bierhaus A (2006). Plasma sRAGE is independently associated with urinary albumin excretion in type 2 diabetes. Diabetes Care; 29(5):1111-3.
27. Humpert PM, Djuric Z, Kopf S, Rudofsky G, Morcos M, Nawroth PP, Bierhaus A (2007). Soluble RAGE but not endogenous secretory RAGE is associated with albuminuria in patients with type 2 diabetes. Cardiovasc Diabetol; 6:9-13.
28. Ji Xiong X, Bi Lin X, Ming Gong Y, Shu Qin L (2003). –429T/C and –374T/A polymorphisms of RAGE gene promoter are not associated with diabetic retinopathy in Chinese patients with type 2 diabetes. Diabetes Care; 26:2696–7.
29. Kalousova M, Ja´ chymova´ M, Mestek O, na Hodkova´M, ta Kazderova´M, Tesarˇ V, Zima T

(2007). Receptor for advanced glycation end products—soluble form and gene polymorphisms in chronic haemodialysis patients. Nephrol Dial Transplant; 22: 2020–6.

30. Kankova K, Zahejsky J, Marova I, Muzik J, Kuhrova V, Blazova M, Znojil V, Beranek M, Vacha J (2001). Polymorphisms in the RAGE gene influence susceptibility to diabetes-associated microvascular dermatoses in NIDDM. J Diabet Comp; 15:185-92.

31. Kankova K, Stejskalova´ A, Hertlova´ M, Znojil V (2005). Haplotype analysis of the RAGE gene: identification of a haplotype marker for diabetic nephropathy in type 2 diabetes mellitus. Nephrol Dial Transplant; 20:1093–102.

32. Katakami N, Matsuhisa M, Kaneto H, Matsuoka T, Sakamoto K, Nakatani Y, Ohtoshi K, Hayaishi-Okano R, Kosugi K, Hori M, Yamasaki Y (2005). Decreased endogenous secretory advanced glycation end product receptor in type 1 diabetic patients: its possible association with diabetic vascular complications. Diabetes Care; 28: 2716–21.

33. Kirbis J, Milutinovic KJ, Steblovnik K, Teran N, Terzic R, Zore M (2004). The –429T/C and –374 T/A gene polymorphisms of the receptor of advanced glycation end products gene (RAGE) are not risk factors for coronary artery disease in Slovene population with type 2 diabetes. Coll Antropol; 28:611–6.

34. Lindholm E, Bakhtadze E, Sjögren M, Cilio CM, Agardh E, Groop L, Agardh C-D (2006). The −374 T/A polymorphism in the gene encoding RAGE is associated with diabetic nephropathy and retinopathy in type 1 diabetic patients. Diabetologia; 49:2745–55.

35. Nakamura K, Yamagishi S, Adachi H, Kurita-Nakamura Y, Matsui T, Yoshida T, Imaizumi T (2007a). Serum levels of sRAGE, the soluble form of receptor for advanced glycation end products, are associated with inflammatory markers in patients with type 2 diabetes. Mol Med; 13:185–9.

36. Nakamura K, Yamagishi S-i, Adachi H, Kurita-Nakamura Y, Matsui T, Yoshida T, Sato A, Imaizumi T (2007b). Elevation of soluble form of receptor for advanced glycation end products (sRAGE) in diabetic subjects with coronary artery disease. Diabetes Metab Res Rev; 23: 368–71.

37. Nakamura K, Yamagishi S-i, Adachi H, Matsui T, Kurita-Nakamura Y, Takeuchi M, Inoue H, Imaizumi T (2008). Serum levels of soluble form of receptor for advanced glycation end products (sRAGE) are positively associated with circulating AGEs and soluble form of VCAM-1 in patients with type 2 diabetes. Microvasc Res; 76:52–6.

38. National Cholesterol Education Program Expert Panel (2001): Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adult (Adult Treatment Panel III). JAMA; 285 (19): 2486-97.

39. Pettersson-Fernholm K, Forsblom C, Hudson BI, Perola M, Grant P, Groop PH (2003). The functional −374 T/A RAGE gene polymorphism is associated with proteinuria and cardiovascular disease in type 1 diabetic patients. Diabetes; 52:891–4.

40. Picheth G, Costantini CO, Pedrosa FO, Martinez TLR, de Souza EM (2007). The -374*A* allele of the receptor for advanced glycation end products (RAGE) gene promoter is a protective factor against cardiovascular lesions in type 2 diabetes mellitus patients. Clin Chem Lab Med; 45(10):1268–72.

41. Pullerits R, Brisslert M, Jonsson IM, Tarkowski A (2006). Soluble receptor for advanced glycation end products triggers a proinflammatory cytokine cascade via beta 2 integrin Mac-1**.** Arthritis Rheum; 54(12):3898-907.

42. Ramprasad S, Radha V, Mathias RA, Majumder PP, Rao MRS, Rema M (2007). Rage gene promoter polymorphisms and diabetic retinopathy in a clinic-based population from South India. Eye; 21: 395–401.

43. Sacks DB (2006). Carbohydrates. In:Burtis CA, Ashwood ER, Bruns DE. Tietz Clinical Chemistry and Molecular Diagnostics, fourth edition, Elsevier Saudders, USA, 837-901.

44. Schmidt AM, Yan SD, Wautier JL, Stern D (1999). Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ Res; 84: 489-97.

45. Tan KC, Shiu SW, ChowWS, Leng L, Bucala R, Betteridge DJ (2006). Association between serum levels of soluble receptor for advanced glycation end products and circulating advanced glycation end products in type 2 diabetes. Diabetologia; 49: 2756–62.

46. Ueda S, Yamagishi S, Takeuchi M, Kohno K, Shibata R, Matsumoto Y, Kaneyuki U, Fujimura T, Hayashida A, Okuda S (2006). Oral adsorbent AST-120 decreases serum levels of AGEs in patients with chronic renal failure. Mol Med; 180: 180–4.

47. Wautier JL, Guillausseau PJ (2001). Advanced glycation end product, their receptors and diabetic angiopathy. Diabetes Metab (Paris); 27:535-42.

48. Wendt T, Tanji N, Guo J, Hudson BI, Bierhaus A, Ramasamy R, Arnold B, Nawroth PP, Yan SF, D'Agati V, Schmidt AM (2003a). Glucose, glycation, and RAGE: implications for amplification of cellular dysfunction in diabetic nephropathy. J Am Soc Nephrol; 14: 1383-95.

49. Wendt TM, Tanji N, Guo J**,** Kislinger TR, Qu W, Lu Y, Bucciarelli LG, Rong LL, Moser B, Markowitz GS, Stein G, Bierhaus A, Liliensiek B, Arnold B, Nawroth PP, Stern DM, D'Agati VD, Schmidt AM (2003b)**.** RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. Am J Pathol; 162:1123–37.

50. Wong TY, Cruickshank KJ, Klein R, Klein BEK, Moss SE, Palta M, Riley WJ, Maclaren NK, Vadheim CM, Rotter JI (2002). HLA-DR3 and DR4 and their relation to the incidence and progression of diabetic retinopathy. Ophthalmol. 109:275–81.

51. Yamamoto Y, Kato I, Doi T, Yonekura H, Ohashi S, Takeuchi M, Watanabe T, Yamagishi S, Sakurai S, Takasawa S, Okamoto H, Yamamoto H (2001). Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. J Clin Invest; 108:261–8.

52. Yan SH, Ramasamy R, Naka Y, Schmidt AM (2003). Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. Circ Res; 93:1159–69.

53. Yokoyama H, Okudaira M, Otani T, Sato A, Miura J, Takaike H, Yamada H, Muto K, Uchigata Y, Ohashi Y, Iwamoto Y (2000). Higher incidence of diabetic nephropathy in type 2 than in type 1 diabetes in early-onset diabetes in Japan. Kidney Int ; 58: 302–11.

54. 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 (2003). 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(3):1097-109.