in vivo *23*: 949-954 (2009)

Associations of –374T/A Polymorphism of Receptor for Advanced Glycation End Products (*RAGE*) Gene in Turkish Diabetic and Non-diabetic Patients with Coronary Artery Disease

OZLEM KUCUKHUSEYIN[1], HULYA YILMAZ AYDOGAN[1], C. SELIM ISBIR[2] and TURGAY ISBIR[1]

[1]*The Institute of Experimental Medical Research, Department of Molecular Medicine, Istanbul University, İstanbul;*
[2]*Department of Cardiovascular Surgery, Marmara University School of Medicine, Istanbul, Turkey*

Abstract. *Background: In this study we aimed to determine the possible risks for the development of coronary artery disease (CAD) in diabetic (DM^+) and non-diabetic (DM^-) patients according to the –374T/A polymorphism of the receptor for advanced glycation end products (RAGE) gene which affects the function of RAGE itself. Materials and Methods: This study was carried out in 52 non-diabetic and 62 diabetic patients with CAD, and 55 CAD-free, healthy volunteers as controls. The A-T transversion polymorphism at position –374 in the promotor region of the RAGE gene was analyzed by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) techniques. Results: The –374T/A AA genotype frequency was statistically higher in the whole patient group when compared with the control group (p=0.034), and statistically higher in the DM^+ group when compared with the control group (p=0.003). Homozygosity for the –374A allele was found to be higher, but not statistically meaningful, in DM^- patients (17.3%) when compared with the control group (13.2%). In this study, in contrast with other studies, we found possesion of the A allele to be an independent risk factor in CAD in patients with diabetes mellitus. Conclusion: Possesion of the –374A allele may contribute to the CAD in diabetic patients with triggering macrophages by increased levels of AGEs.*

Correspondence to: Professor Dr. Turgay Isbir, Director of Molecular Medicine, The Institute of Experimental Medical Research, Department of Molecular Medicine, Istanbul University, Capa, 34390 İstanbul, Turkey. Tel/Fax: +90 2126351959, e-mail: tisbir@superonline.com

Key Words: RAGE, coronary artery disease, diabetes mellitus, polymorphism, genetics.

Atherosclerotic coronary artery disease, by the interaction of multiple genetic and environmental risk factors, is a complex disease and a major cause of morbidity and mortality in the Western population (1-4). Diabetes mellitus is the most prevalent metabolic syndrome which is characterized by hyperglycaemia, resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes (5). Such chronic hyperglycemia results in the formation and accumulation of irreversible advanced glycation end products (AGEs) that have a plethora of adverse effects, resulting in dysfunction of the vasculature and effects shown to be mediated by specific AGE binding and degrading receptors (receptor for AGEs, RAGE) which are present in the non-diabetic population, but are two- to five-fold higher in diabetics (5-12).

Various lines of evidence suggest that genetic factors contribute significantly to the risk of CAD (1, 2, 13). The *RAGE* gene has been proposed as having a role in diabetic atherosclerosis (14). Its gene product, 35 kDa polypeptide 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 (12, 15-17). The *RAGE* gene is located on chromosome 6p21.3 in the major histocompatibility complex and has 11 exons (15, 16).

The hyperglycaemic state seen in diabetes mellitus is associated with the development of diabetes-specific microvascular complications (retinopathy, nephropathy and neuropathy) and accelerated macrovascular disease (atherosclerosis leading to heart disease, stroke and peripheral vascular disease). Evidence implicates the formation and subsequent effects of AGEs as a contributing cause. AGEs have a number of deleterious effects, including cross-linking of proteins, modification of matrix components, platelet aggregation, defective vascular relaxation, abnormal lipoprotein metabolism, and induction of cytotoxic pathways that may affect the pathophysiology of the vascular wall.

AGE-specific cellular receptor (RAGE) complexes identified on different cell types, including endothelial cells, smooth muscle cells, lymphocytes, and monocytes, where they mediate AGE removal as well as multiple biological responses, including the triggering of proinflammatory and procoagulatory genes and cellular migration and proliferation. Accumulation of AGE/RAGE can be seen at sites of vascular disease in both animal models of diabetes and human diabetics (5-11, 18, 19).

The concept RAGE contributes to the development of coronary atherosclerosis is gaining favour. Several polymorphic sites have so far been found within the *RAGE* gene (13, 15). A functional –374T/A polymorphism in the promoter of the *RAGE* gene which has been associated with vascular complications has been shown to exert significant effects on transcriptional activity. Investigation of this common variant revealed that the introduction of the –374A allele abolished a nuclear protein binding site, supporting the role of this polymorphism in affecting *RAGE* transcriptional repression. The –374T/A variant has thus become a topic of great interest (13, 14, 20).

Accordingly, this study was to determine the role of this polymorphism in CAD in diabetic and non-diabetic Turkish patients.

Materials and Methods

Study participants and clinical investigation. The *RAGE* –374T/A gene polymorphism was studied in 52 non-diabetic (DM^-) [11 (21.2%) women, 41 (78.8%) men] and 62 diabetic (DM^+) [26 (41.9%) women, 36 (58.1%) men] cases with CAD.

Patients with severe coronary vascular disease were documented by angiography. Angiographic inclusion criteria were: ≥50% stenosis of at least one major coronary vessel because of atherosclerosis, and a vascular event, defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting. Patients were included irrespective of concomitant risk factors for atherosclerosis such as smoking, arterial hypertension and diabetes mellitus. Participants were of both sexes and the height and weight of each were also recorded, thus permitting calculation of the body mass index (BMI): [weight (kg)/height^2 (m)]×100.

Healthy persons [27 (49.1%) women, 28 (50.9%) men] without any symptoms of diabetes mellitus and CAD were selected for the control group. Coronary angiography was not performed on these individuals, and therefore the presence of atherosclerotic coronary arteries could not be excluded. However, none of these individuals had any history of any vascular event.

Polymerase chain reaction (PCR)-based detection of RAGE mutation. Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with a salting-out procedure (21).

RAGE –374T/A genotype was analysed by PCR-restriction fragment length polymorphism. The –374T/A mutation detected by cutting the PCR product with the restriction endonuclease Tsp509 I (New England Biolabs, Beverly, MA, USA). The digested DNAs were separated on 3% agarose gel in 1XTris borate EDTA buffer followed by staining with ethidium bromide solution (13, 20, 22-24). The RAGE genotypes were typed by visualization under ultraviolet light photographed using a Polaroid camera.

Statistical methods. Statatistical analysis was performed by using SPSS software package (revision 11.5 SPSS Inc., Chicago, IL, USA). Clinical laboratory data are expressed as mean±SD. Mean values were compared between patients and controls by unpaired Student's *t*-test. Differences in the distribution of *RAGE* –374 T/A genotypes and alleles between cases and controls were tested using the Chi-square statistic. Allele frequencies were estimated by gene counting methods. Values of $p<0.05$ were considered statistically significant.

Results

Demographic characteristics are summarised in Table I. DM^- CAD, DM^+ CAD patients and the control group were compared with advanced chi-square analysis. In DM- patients, there was a higher ratio of male gender ($p=0.008$), higher levels of total cholesterol ($p=0.053$), smoking ($p=0.001$) and less frequent family history of CAD ($p=0.024$). In DM^+ patients, higher systolic ($p=0.064$) and diastolic pressures ($p=0.019$), and a higher prevalance of hypertriglyceridemia ($p=0.014$) were found. When the disease-specific complications, such as left ventricular hypertrophy (LVH), left atrium dilatation (LAD) and hypertension, were compared, there was no significance for LVH and LAD but hypertension was significantly more frequent in the DM^+ group ($p=0.001$).

We found a higher ratio of male gender ($p=0.037$), higher systolic ($p=0.058$) and diastolic pressures ($p=0.013$), and higher prevalance of hypertriglyceridemia ($p=0.044$) in the patient group (DM^+ and DM^- CAD) when compared with the control group (Table I).

The frequency of the wild-type T allele in the control group was statistically higher ($p=0.034$) when compared with the whole patient group, while the AA genotype frequency was statistically higher in the whole patient group *versus* controls (28.3% *vs*. 13.2%). Moreover, when compared with DM^+ CAD patients, the T allele frequency was statistically higher ($p=0.003$) in the control group, and the AA genotype frequency was significantly lower (13.2% *vs*. 38.9%) (Table II).

There was no statistically assosiation between *RAGE* –374T/A polymorphism and cardiovascular risk factors in the whole patient group ($p>0.05$).

In DM^+ CAD patients, serum triglyceride ($p=0.007$) and VLDL-cholesterol ($p=0.01$) levels were statistically higher in individuals with the TT genotype than in those with the AA or AT genotype.

DM^- patients with AA or AT genotype had a significantly higher ratio of male gender ($p=0.028$) (Table III) and a trend for a smoking habit ($p=0.054$) (Table IV)

Table I. *Demographic and biochemical characteristics of the study population.*

	Control	CAD Patients		*P*-value[1]	Total CAD	*P*-value[2]
		DM⁻CAD	DM⁺CAD			
Gender (male/female) (n)	27 (49.1%)/28 (50.9%)	11 (21.2%)/41(78.8%)	26 (41.9%)/36 (58.1%)	0.008	37 (32.5%)/77 (67.5%)	0.037
Age (years)	57.96±12.63 (n=55)	58.42±10.34 (n=52)	61.42±8.5 (n=62)	0.158	60.05±9.472 (n=114)	0.280
Body mass index (kg/m2)	25.52±5.63 (n=50)	25.81±3.42 (n=50)	27.48±5.10 (n=55)	0.080	26.68±4.44 (n=105)	0.202
Systolic pressure (mmHg)	123.60±17.11 (n=50)	127.30±26.82 (n=50)	135.59±26.46 (n=36)	0.064	130.81±26.84 (n=86)	0.058
Diastolic pressure (mmHg)	76.20±10.85 (n=50)	79.80±14.21 (n=50)	85.13±18.10 (n=36)	**0.019**	82.03±16.07 (n=86)	**0.013**
Total-cholesterol (mg/dl)	192.30±67.91 (n=33)	220.44±56.22 (n=50)	196.86±46.41 (n=36)	0.053	210.57±53.34 (n=86)	0.171
Triglycerides (mg/dl)	145.52±87.53 (n=33)	145.82±91.34 (n=50)	158.23±116.04 (n=35)	0.819	150.93±101.76 (n=85)	0.788
LDL-cholesterol (mg/dl)	134.25±60.67 (n=32)	133.26±37.57 (n=50)	117.29±33.33 (n=34)	0.196	126.80±36.57 (n=84)	0.519
HDL-cholesterol (mg/dl)	36.81±16.26 (n=32)	44.06±12.79 (n=50)	41.79±13.38 (n=35)	0.076	43.13±13.01 (n=85)	0.055
VLDL-cholesterol (mg/dl)	28.04±17.99 (n=27)	25.88±13.91 (n=43)	32.91±28.39 (n=33)	0.335	28.93±21.55 (n=76)	0.847
LVH (%)	-	11 (25.6%)	22 (41.5%)	0.102	33(34.4%)	-
LAD (%)	-	5 (23.8%)	4 (33.3%)	0.690	9(27.3%)	-
Hypertension (%)	-	15 (30.0%)	39 (62. %9)	**0.001**	54 (48.2%)	-
Family history of CAD (%)	10 (58.8%)	13 (26.5%)	27 (47.4%)	**0.024**	50 (40.7%)	0.100
Hypercholesterolemia (%)	15 (45.5%)	32 (64.0%)	37 (66.1%)	0.128	69 (65.1%)	**0.044**
Hypertriglyceridemia (%)	13 (39.4%)	22(44.0%)	37(67.3%)	**0.014**	59(56.2%)	0.092
Smoking (%) (Yes)	7 (58.8%)	37 (77.1%)	24 (40.7%)	**0.001**	61 (57.0%)	0.223

The results are shown as mean±SD. DM⁺: Diabetic patients, DM⁻: non-diabetic patients, CAD: coronary artery disease, n: number of individuals, [1]*p*-value of DM⁻ and DM⁺ *vs*. control group seperately, [2]*p*-value of total patient group *vs*. control group.

when compared with those with the TT genotype. When the individual and common effects of environmental factors and smoking habit in the development of CAD as a genetic factor was analyzed, in DM⁻ CAD patients having either A or T allele and smoking habit, the risk was lower. Therefore, our findings shows no relationship between smoking habit and –374T/A polymorphism in the development of CAD (Table IV). Like smoking habit, the possible risks in the development of CAD, the individual and common effects of being male in gender and having either A or T allele is shown in Table III. In DM⁺ patients, *RAGE* –374T/A polymorphism and both being male in gender and having a smoking habit had no effect on the odds ratio for the development of CAD.

In the controls together with AA and AT genotype, the systolic (130.46±29.79 *vs*. 121.66±20.07) and diastolic (81.40±15.30 *vs*. 76.94±11.89) blood pressures were higher in those with the TT genotype but the difference was not statistically meaningful.

Table II. *Genotype and allele frequencies of RAGE –374T/A gene in study groups.*

	Controls (N=53)	DM⁻CAD (N=52)	DM⁺CAD (N=54)	Total patient (N=106)
Genotypes				
AA	7 (%13.2)	9 (%17.3)	21 (%38.9)**	30 (%28.3)*
TT	21 (%39.6)	19 (%36.5)	14 (%25.9)	33 (%31.1)
AT	25 (%47.2)	24 (%46.2)	19 (%35.2)	43 (%40.6)
Alleles				
A	39 (%36.79)	42 (% 40.38)	61 (%56.48)	103 (%48.58)
T	67 (%63.20)	62 (%59.61)	47 (%43.51)*	109 (%51.41)

Chi-square test was used to compare genotypes in the study group. *$p<0.05$, **$p<0.01$.

Discussion

Coronary artery disease is a multifactorial disease in which genetic and environmental factors play an important role. These factors may be different in each race and ethnic group (3, 4, 25, 26).

Protein glycation is the most important process after synthesis (27) and the biological activity of sugar, such as differentiation, development and growth, covers a wide range (28-32). Non-enzymatic glycolysation, which leads to the formation of AGEs has many adverse effects and depends on the concentration of glucose; therefore, in diabetes mellitus increased glycolysation levels are detected which are responsible for the chronic complications of diabetes (6, 19). AGEs show their effects in extracellular matrix, in vasculature and by binding to their receptors (RAGE). Because of their prothrombotic and proinflammatory effects, the single nucleotide polymorphisms of the *RAGE* gene are gaining importance as being a candidate gene for atherosclerosis and other associated diseases.

Table III. *The individual and common effects of male gender and –374 T or –374 A allele on the odds ratio of the possible risks for the development of CAD in non-diabetic patients.*

DM– CAD/ Control	Male gender	–374 TT+AT genotype (T allele)	Male gender and –374 T allele	–374 AA+AT genotype (A allele)	Male gender and –374 A allele
OR	3.594	0.727	4.080	1.140	3.267
95% CI	1.536-8.409	0.249-2.123	0.441-37.783	0.518-2.507	0.832-12.828

OR: Odds ratio, CI: confidental interval.

Table IV. *The individual and common effects of smoking habit and –374 T or –374 A allele on the odds ratio of the possible risk for the development of CAD in non-diabetic patients.*

DM– CAD/ Control	Smoking habit	–374 TT+AT genotype (T allele)	Smoking habit and –374 T allele	–374 AA+AT genotype (A allele)	Smoking habit and –374 A allele
OR	4.805	0.727	0.961	1.140	0.986
95% CI	1.481-15.595	0.249-2.123	0.909-1.016	0.518-2.507	0.207-4.692

OR: Odds ratio, CI: confidental interval.

Table V. *Comparison of previous studies of the RAGE –374T/A genotypes and cardiovascular diseases.*

Author	Year	Race	Polymorphism	Outcome
Studies reporting a positive association				
Falcone *et al*. (24)	2008	Italian	–374T/A	In non-diabetic patients having this polymorphism (A allele) has a protective role for developing CAD.
Falcone *et al*. (20)	2005	Italian	–374T/A	AA genotype is protective in cardiac events, such as vascular stenosis, and diabetes.
Falcone *et al*. (22)	2004	Italian	–374T/A	A allele has a protective effect in CAD.
Pettersson-Fernholm *et al*. (33)	2003	Finnish	–374T/A	In CAD, acute MI and peripheral vascular disease patients, A allele is protective.
Hudson *et al*. (14)	2001	English	–374T/A	A allele down-regulates the transcription of RAGE in diabetic retinopathy patients.
Santos *et al*. (38)	2005	Africans/ White Brazilians	–374T/A	This polymorphism decreases the risk of ischemic hearth disease in African-Brazilians, but no association in White Brazilians.
Zee *et al*. (39)	2006	Caucasian	–429T/C –374T/A G82S	The patients carrying –374T>A variant have less ratio of ischemic stroke
Studies reporting negative association				
Hudson *et al*. (34)	2001	English	–374T/A	No association between diabetic and non-diabetic macrovascular diseases and –374/A polymorphism
Kirbis *et al*. (35)	2004	Slovenian	–374T/A and –429T/C	No association with diabetic CAD
Jixiong *et al*. (36)	2003	Chinese	–374T/A and –429T/C	No association with diabetic retinopathy.

CAD: Coronary heart disease, CAD: Coronary artery disease, MI: Myocardial infarction, NIDDM: Non-insulin dependent diabetes mellitus.

We investigated associations of *RAGE* polymorphism with the risk of CAD with or without diabetes mellitus in a case-control based study in a Turkish population. Our study is the first study to examine these associations. The causal role of RAGE in the pathogenesis of CAD has been examined in several studies (Table V). In this study, we observed an association between –374T/A polymorphism and diabetic CAD as a independent risk factor for the development of the disease and no association between –374T/A polymorphism and non-diabetic CAD or the

whole patient group. These findings were similar to those of population studies but not all (Table V).

Our findings of an association of RAGE –374T/A polymorphism with diabetic and non-diabetic CAD patients differed from some of the studies shown in Table V. Pettersson-Fernholm *et al*. (33) and Falcone *et al*. (20, 22, 24) found that because the conversion of T→ A caused the repression of receptor expression, resulting in inefficiency in cellular signalling, the A allele had a protective effect for the development of CAD. However Hudson *et al*. (34), Kirbis *et al*. (35) and Jixiong *et al*. (36) suggested no association between A allele and vascular diseases (Table V). The difference between those studies and our study could be because of the size of the study groups and ethnic origins of the population and/or differences in the smoking habit and the other risk factors for CAD.

In this study, we found the possession of the A allele to be an independent risk factor for CAD. The repression of RAGE expression due to the A allele results in higher blood levels of deleterious AGEs, HbA1c, glycated-LDL and glycated collagen. The contribution of the cross-linking of AGEs with vessel proteins that causes the thickening and leakeage of the wall in vascular diseases are well defined (37).

In conclusion, our results demonstrated that in non-diabetics, being male in gender and having a smoking habit were the most important risk factors for CAD, and in diabetics, the AA genotype had more significance than the other variants for the development of CAD. As such, larger numbers of study groups are required to establish whether –374T/A polymorphism has a causative role in the pathogenesis of CAD.

Acknowledgements

This present work was supported by the Research Fund of Istanbul University, project number: T-967/06102006.

References

1 Gerszten RE and Rosenzweig A: Coronary Atherosclerosis. *In*: Principles of Molecular Medicine. Chicago; Humana Press and Jameson JL (ed.). 1998.
2 Novelli G, Borgiani P, Giardina E, Mango R, Contino G, Romeo F and Mehta JL: Role of genetics in prevention of coronary atherosclerosis. Curr Opin Cardiol *18*: 368-371, 2003.
3 Ross R: The pathogenesis of atherosclerosis. *In*: Braunwald Heart Disease A Textbook of Cardiovascular Medicine, 4th edition, An HBJ International Edition. Philadelpia: Saunders *1(36)*: 1106-1124, 1992.
4 Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature *362*: 801-809, 1993.
5 Ramasamy R, Vannucci SJ, Yan DSS, Herold K, Yan SF and Schmidt AM: Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration and inflammation. Glycobiol *15(7)*: 16R-28R, 2005.
6 Baldwin JS, Lee L, Leung TK, Muruganandam A and Mutus B: Identification of the site of non-enzymatic glycation of glutathione peroxidase: rationalization of the glycation-related catalytic alterations on the basis of three-dimensional protein structure. Biochim Biophys Acta *1247*: 60-64, 2005.
7 Simm A, Casselmann C, Schubert A, Hofmann S, Reimann A and Silber RE: Age-associated changes of AGE-receptor expression: RAGE up-regulation is associated with human heart dysfunction. Experimental Gerontology *39*: 407-413, 2004.
8 Wautier JL and Schmidt AM: Protein glycation: A firm link to endothelial cell dysfunction. Circ Res *95*: 233-238, 2004.
9 Forbes JM, Soldatos G and Thomas MC: Below the radar: Advanced glycation end products that detour around the side. Clin Biochem Rev *26*: 123-134, 2005.
10 Baynes JW and Thorpe SR: Glycoxidation and lipoxidation in atherogenesis. Free Radic Biol Med *28(12)*: 1708-1716, 2000.
11 Bierhaus A, Hoffman MA, Ziegler R and Mawroth PP: AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus–The AGE concept. Cardiovasc Res *37*: 586-600, 1998.
12 Singh R, Barden A, Mori T and Beilin L: Advanced glycation end products: A review. Diabetologia *44*: 129-146, 2001.
13 Yan SF, Ramasamy R, Naka Y and Schmidth AM: Glycation, inflammation, and RAGE. A scaffold for the macrovascular complications of diabetes and beyond. Circ Res *93*: 1159-1169, 2003.
14 Hudson BI, Stickland MH, Futers TS and Grant PJ: Effects of novel polymorphisms in the RAGE gene on transcriptional regulation and their association with diabetic retinopathy. Diabetes *50*: 1505-1511, 2001.
15 Hudson B, Hofmann M, Bucciarelli L, Wendt T, Moser B, Lu Y, Qu W, Stern D, D'Agati V, Yan S, Grant and Schmidt A: Glycation and diabetes: The RAGE connection. Curr Sci *83(12)*: 1515-1521, 2002.
16 Li J and Schmidt AM: Characterization and functional analysis of the promote of *RAGE*, the receptor for advanced glycation end products. Biol Chem *273(26)*: 16498-16506, 1997.
17 Hudson B, Wendt T, Bucciareli L, Rong L, Naka Y, Yan S and Schmidt A: Diabetic vascular disease: It's all RAGE: Forum review. Antiox Red Signal *7(11-12)*: 1588-1600, 2005.
18 Szweda PA, Friguet B and Szweda LI: Proteolysis, free radicals and aging. Free Radic Biol Med *33*: 29-36, 2002.
19 Adachi T, Ohta H, Hirano K, Hayashi K and Marklund SL: Non-enzymic glycation of human extracellular superoxide dismutase. Biochem J *279*: 263-267, 1991.
20 Falcone C, Campo I, Emanuele E, Buzzi MP, Geroldi D, Belvito C, Zorzetto M, Sbarsi I and Cuccia M: –374T/A polymorphism of the *RAGE* gene promoter in relation to severity of coronary atherosclerosis. Clinica Chimica Acta *354*: 111-116, 2005.
21 Miller SA, Dykes DD and Polesky HF: A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res *16*: 1215, 1998.
22 Falcone C, Campo I, Emanuele E, Buzzi MP, Zorzetto M, Sbarsı I and Cuccıa M: Relationship between the –374T/A *RAGE* gene polymorphism and angiographic coronary artery disease. Int J Mol Med *14*: 1061-1064, 2004.
23 Kankova K, Zahejsky J, Marova I, Muzik J, Kuhrova V, Blazkova M, Znojil V, Beranek M and Vacha J: Polymorphisms in the *RAGE* gene influence susceptibility to diabetes-associated microvascular dermatoses in NIDDM. J Diabetes Compl *15*: 185-192, 2001.

24 Falcone C, Geroldi D, Buzzi MP, Emanuele E, Yılmaz C, Fontana JM, Vignali L, Boiocchi C, Sbarsi I and Cuccia M: The –374 T/A *RAGE* gene polymorphism protects against future cardiac events in nondiabetic patients with coronary artery disease. Arch Med Res *39*: 320-325, 2008.

25 Bolli G, DeFeo P, Compagnucci P, Angeletti G, Sansteusanio F, Brunetti P and Gerich J: Abnormal glucose counter regulation in insulin dependent diabetes mellitus: Interaction of anti-insulin antibodies and impaired glucagon and norepinephrine secretion. Diabetes *32*: 134, 1983.

26 Foster DW. Fauci AS, Braunwald E, Iselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL and Longo DL: Diabetes mellitus. *In*: Harrison's Principles of Internal Medicine, 14th ed. New York: The McGraw-Hill Companies Inc. pp: 2060-2080, 1998.

27 Kobata A: Structures and functions of the sugar chains of glycoproteins. Eur J Biochem *209*: 483-501, 1992.

28 Allen HJ and Kisailus EC: Glycoconjugates: Composition, Structure and Function. New York: Marcel Dekker Inc. pp. 685, 1992.

29 Feizi T: Cell-cell adhesion and membrane glycosylation. Curr Opin Struc Biol *1*: 766-770, 1991.

30 Varki A: Biological roles of oligosaccharides. *In*: Tools for Glycobiology. Novato: Glyco Inc. 7.18-7.32, 2001.

31 Pollard TD and Earnshaw WC: Cell Biology. Philadelphia: Saunders. pp. 316, 2004.

32 Nelson DL and Cox MM: Principles of Biochemistry. New York: Lehninger. pp. 261-268, 2005.

33 Pettersson-Fernholm K, Forsblom C, Hudson BI, Perola M and Grant PJ: The functional –374T/A *RAGE* gene polymorphism is associated with proteinuria and cardiovascular disease in type 1 diabetic patients. Diabetes *52(3)*: 891-894, 2003.

34 Hudson BI, Sticland MH, Futers TS and Grant PJ: Study of the -429 T/C and –374 T/A receptor for advanced glycation end products promoter polymorphisms in diabetic or nondiabetic subjects with macrovascular disease. Diabetes Care *24(11)*: 2004, 2001.

35 Kirbis J, Milutinović A, Steblovnik K, Teran N, Terzić R and Zorc M: The -429 T/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(2)*: 611-616, 2004.

36 Jixiong X, Bilin X, Minggong Y and Shuqin L: -429 T/C and –374 T/A polymorphisms of *RAGE* gene promoter are not associated with diabetic retinopathy in Chinese patients with type 2 diabetes. Diabetes Care *26(9)*: 2696-2697, 2003.

37 Bohlender JM, Franke S, Stein G and Wolf G: Advanced glycation end products and the kidney. Am J Physiol Renal Physiol *289*: 645-659, 2005.

38 Santos KG, Canani LH, Gross JL, Tschiedel B, Souto KEP and Roisenberg I: 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 Gen Metab *85*: 149-156, 2005.

39 Zee RYL, Romero JR, Gould JL, Ricupero DA and Ridker PM: Polymorphisms in the advanced glycosylation end product-specific receptor gene and risk of incident myocardial infarction or ischemic stroke. Stroke *37*: 1686-1690, 2006.

Received June 17, 2009
Revised August 10, 2009
Accepted September 21, 2009