Relation Between Development of Nephropathy and the p22phox C242T and Receptor for Advanced Glycation End Product G1704T Gene Polymorphisms in Type 2 Diabetic Patients

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OBJECTIVE — The development of diabetic nephropathy is considered to be associated with oxidative stress. NADPH oxidase and the receptor for advanced glycation end products (RAGE) have attracted attention as mechanisms of generating oxidative stress. We studied the relation between the genotypes of the NADPH p22phox C242T and RAGE G1704T polymorphisms and the development of diabetic nephropathy in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — Using a retrospective review of clinical data, we allocated 181 Japanese type 2 diabetic patients to one of two groups: patients without diabetic nephropathy (group N; $n = 108$) and patients developing diabetic nephropathy (group D; $n = 73$) for 10 years or more. The p22phox C242T and RAGE G1704T polymorphisms were examined by Taqman PCR methods.

RESULTS — The frequency of the p22phox CC genotype was significantly higher in group D than in group N (90 vs. 79%; $P = 0.0427$). The frequency of the RAGE GT + TT genotype was significantly higher in group D than in group N (26 vs. 13%; $P = 0.0313$). The frequency of the combination of p22phox CC and RAGE GT + TT genotypes was significantly higher in group D than in group N (22 vs. 8%; $P = 0.0057$). In multiple logistic regression analysis, systolic blood pressure, HbA_{1c}, triglycerides, and the combination of polymorphisms were shown to be independent variables.

CONCLUSIONS — These results suggest that assessment of the combination of NADPH p22phox C242T and RAGE G1704T polymorphisms may be useful in identifying the risk for developing diabetic nephropathy in type 2 diabetic patients.

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Diabetic nephropathy is the main cause of end-stage renal disease. Although poor glycemic control for a long period of time is a major factor in the development of diabetic nephropathy, hypertension, hyperlipidemia, and genetic factors are also associated with its development. The molecular mechanisms that underlie the pathogenesis of diabetic nephropathy remain unclear. Recent studies have highlighted the hyperglycemia-induced production of reactive oxygen species (ROS) as one of the mechanisms involved in the development of diabetic microangiopathy, via the following pathological pathways: activation of protein kinase C (PKC), formation of advanced glycation end products (AGEs), and activation of transcription factors such as nuclear factor-κB (NF-κB) (1). It has been proposed that genes associated with oxidative stress contribute to the risk of diabetic nephropathy (2–4). Recently, NADPH oxidase and the receptor for AGEs (RAGE) have received attention as mechanisms of generating oxidative stress. NADPH oxidase is a membrane-associated enzyme for superoxide production in vascular smooth muscle cells and endothelial cells (5). It has been reported that the NADPH oxidase p22phox C242T polymorphism is associated with vascular superoxide production in human blood vessels from coronary artery disease (CAD) patients (6). In addition, it has been suggested that AGEs might play a role in the pathogenesis of diabetic nephropathy and the progression to renal failure (7). The actions of AGEs are dependent on RAGE; thus, the AGE-RAGE system might participate in the development of diabetic microangiopathy (7). An intron polymorphism in the RAGE gene, G1704T, has been reported to be associated with microvascular dermatoses and antioxidant status (8,9). Moreover, a recent study has demonstrated that AGE-induced generation of ROS involves AGE-RAGE interaction through stimulation of membrane-bound NADPH oxidase (10). Therefore, in this study, we decided to investigate the association of genotypes of the p22phox C242T and RAGE G1704T

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Abbreviations: AGE, advanced glycation end product; CAD, coronary artery disease; NF-κB, nuclear factor-κB; PKC, protein kinase C; RAGE, receptor for AGEs; ROS, reactive oxygen species; UAI, urinary albumin index.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

polymorphisms and the combination of these polymorphisms with the susceptibility to developing nephropathy in Japanese type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

— The subjects were 691 unrelated Japanese patients diagnosed with type 2 diabetes according to World Health Organization criteria (11) who had been followed up on a regular basis at the outpatient clinic of Saitama Social Insurance Hospital since 1990. From among these subjects, we recruited all the patients who fulfilled the noted criteria (n = 181) and allocated them to one of two groups: group N, comprised of 108 patients without diabetic nephropathy, and group D, comprised of 73 patients with developing diabetic nephropathy. The stage of nephropathy was defined according to urinary excretion of albumin per gram of creatinine (urinary albumin index; UAI) and categorized as follows: normoalbuminuria, <20 mg/gCr, stage 0; microalbuminuria, 30–300 mg/gCr, stage 1; and macroalbuminuria, >300 mg/gCr, stage 2. Diabetic patients with UAI of 21–29 mg/gCr were assumed to be "borderline" and were therefore excluded from this study. Group N was defined using the following criteria: *1*) >10 years after diagnosis of type 2 diabetes and *2*) normoalbuminuria throughout the follow-up period. Group D was defined using the following criteria: *1*) normoalbuminuria (stage 0) or microalbuminuria (stage 1) at the beginning of follow-up and *2*) progression from normoalbuminuria (stage 0) to microalbuminuria (stage 1) or macroalbuminuria (stage 2), or progression from macroalbuminuria (stage 1) to macroalbuminuria (stage 2) during the follow-up period. To exclude patients with nondiabetic renal disease, patients with microscopic hematuria, clinical pyuria, or evidence of bacterial infection in a urine culture were excluded from this study. Diabetic retinopathy was diagnosed by independent diabetic ophthalmologists using standard fundus photographs at least every year; the stage of diabetic retinopathy was classified as simple, preproliferative, or proliferative retinopathy. HbA_{1c} levels, lipid profiles, blood pressure, and urinary excretion of albumin were measured at least every year. Clinical data, including onset age, BMI at the diagnosis of diabetes, disease duration since the diagnosis of type 2 diabetes, HbA_{1c} levels, blood pressure, lipid profiles, and the use of medication were obtained from the medical records of the patients. Written informed consent was obtained from each subject after full explanation of the purpose, nature, and risk of all procedures used. The protocol of this study was approved by the ethical review committee of the Saitama Social Insurance Hospital, Saitama, Japan.

Table 1—*Characteristics of patient groups*

	Group N	Group D	*P*
n	108	73	—
Sex (M/F)	52/56	39/34	NS
Onset age (years)	44.3 ± 7.2	45.2 ± 8.2	NS*
Disease duration (years)	18.4 ± 5.4	18.5 ± 5.5	NS*
BMI at onset (kg/m^2)	23.4 ± 3.7	24.0 ± 3.2	NS
HbA_{1c} (%)	7.3 ± 1.1	8.0 ± 1.2	0.0002
Total cholesterol (mmol/l)	5.11 ± 0.73	5.15 ± 0.78	NS
Triglyceride (mmol/l)	1.25 ± 0.73	1.53 ± 0.97	0.0137*
LDL cholesterol (mmol/l)	3.08 ± 0.71	3.06 ± 0.74	NS
Systolic blood pressure (mmHg)	134.0 ± 15.2	149.6 ± 18.0	<0.0001
Diastolic blood pressure (mmHg)	81.2 ± 8.8	86.2 ± 9.5	0.0004
Retinopathy (%)	43	67	0.0014
Medication (diet/OHA/insulin)	43/36/29	9/29/34	NS

Data are means ± SD unless otherwise indicated. **P* values determined by Mann Whitney *U* test. OHA, oral hyperglycemic agents.

DNA preparation and detection of polymorphisms in p22phox and RAGE

Genomic DNA was isolated from peripheral blood leukocytes of the 181 patients, as previously described (12). The C242T transition polymorphism in the NADPH oxidase p22phox gene (GenBank M61107), exon 6 region, was determined by the TaqMan (Applied Biosystems, Tokyo, Japan) PCR method (13). The following primers and probes were included in the reaction: forward primer, 5′-TTC CTC CCT CCC CCA GG-3′; reverse primer, 5′-CCT GGT AAA GGG CCC GAA-3′; T allele−specific probe, 5′-Vic-ACA GAA G*T*A CAT GAC CG-3′; and C allele−specific probe, 5′-Fam-AGA AG*C* ACA TGA CCG-3′. The G1704T transversion polymorphism in the RAGE gene (GenBank D28769), intron region, was also determined by TaqMan PCR method. The following primers and probes were included in the reaction: forward primer, 5′-AGG ATG TGA GTG ACC TGG AGA GA-3′; reverse primer, 5′-TCA GCT CCT AGC CTG CCT TTC-3′; T allele−specific probe, 5′-Vic-CCA TAA CTA *T*CA ACA GGG-3′; and G allele−specific probe, 5′-Fam-CAT AAC TA*G* CAA CAG GG-3′. PCR was carried out using an ABI Prism 7700 (Applied Biosystems).

Statistical analysis

The sample size of this study was established on the basis of our pilot study. Regarding p22phox, our pilot studies indicated that the frequency of 242CT + TT would be 0.10 for Group D and 0.28 for Group N. It was calculated that the number of subjects needed to achieve 80% power to detect a difference between the two groups, with a significance level α (chance of a two-sided α error) of 0.05 was 59. Regarding RAGE, pilot studies indicated that the frequency of 1704GT + TT would be 0.30 for Group D and 0.13 for Group N. It was calculated that the number of subjects needed to achieve 80% power to detect a difference between the two groups was 72.

Data are expressed as means ± SD. The statistical significance of differences in mean values was analyzed by Student's *t* test or the Mann-Whitney *U* test. The frequencies of various alleles and genotypes were compared between the groups by χ^2 or Fisher's exact test, and multiple logistic regression analysis was performed to calculate the odds ratio and to evaluate the relation between the genotypes and other conventional risk factors. All statistical analyses were performed using Statview Software (version 5.0 for Windows; SAS Institute, Cary, NC).

RESULTS

— Table 1 shows the clinical

Table 2—*Frequencies of genotypes of p22phox C242T and RAGE G1704T polymorphisms*

	Group N	Group D	χ^2	*P*
p22phox242				
CC	85 (79)	66 (90)		
CT + TT	23 (21)	7 (10)	4.318	0.0427
RAGE1704				
GG	94 (87)	54 (74)		
GT + TT	14 (13)	19 (26)	4.987	0.0313

Data are *n* (%) unless otherwise indicated.

characteristics of the enrolled patients. Clinical data, including HbA_{1c} levels, lipid profiles, blood pressure, and the use of medication were obtained from the medical records, and retinopathy was assessed in 2001. There were no significant differences in age at onset, disease duration since the diagnosis of type 2 diabetes, BMI at onset, total or LDL cholesterol, or the use of medication between groups N and D, whereas blood pressure, HbA_{1c} levels, triglyceride levels, and the proportion of subjects with retinopathy were significantly higher in group D than in group N.

The genotype distribution of these genes in each group is shown in Table 2. The genotype frequencies in both groups were in Hardy-Weinberg equilibrium. The frequency of the CC, CT, and TT genotypes of the p22phox gene were 79, 20, and 1% in group N compared with 90, 8, and 2% in group D, respectively. The allelic frequencies of the C and T alleles were 95 and 5% in group D versus 89 and 11% in group N, respectively. Because the frequency of the TT genotype was low, we divided the enrolled subjects into two groups: CC and CT + TT. The frequency of the CT + TT genotypes was significantly higher in group N than in group D ($\chi^2 = 4.318$, $P = 0.0427$; 2 × 2). In addition, multiple logistic regression analysis identified the HbA_{1c} level, systolic blood pressure, and triglyceride level as independent risk factors for the development of diabetic nephropathy ($P < 0.005$) within the following variables: age at onset, disease duration, BMI at onset, HbA_{1c}, total cholesterol, triglyceride, retinopathy, systolic blood pressure, and p22phox polymorphism; this was not the case for p22phox CT + TT ($P = 0.0991$) (data not shown). The frequencies of the GG, GT, and TT genotypes of the RAGE G1704T gene were 87, 12, and 1%, respectively, in group N compared with 74, 23, and 3% in group D. The allelic frequencies of the G and T alleles were 74 and 26% in group D versus 87 and 13% in group N, respectively. Because the frequency of TT was low, we divided the enrolled subjects into two groups: GG and GT + TT. The frequency of the GT + TT genotypes was significantly higher in group D than in group N ($\chi^2 = 4.987$, $P = 0.0313$; 2 × 2). Multiple logistic regression analysis identified HbA_{1c} level and systolic blood pressure as independent risk factors for the development of diabetic nephropathy ($P < 0.005$), whereas this was not the case for RAGE GT + TT ($P = 0.0522$) (data not shown).

Next, we analyzed the relation between the development of diabetic nephropathy and the combination of these gene polymorphisms in the patients (Table 3). According to the results in Table 2, we assigned the combination of these genotypes to three levels of predicted risk of developing diabetic nephropathy: high-risk genotype (p22phox CC and RAGE GT + TT), intermediate-risk genotypes (p22phox CC and RAGE GG, p22phox CT + TT and RAGE GT + TT), and low-risk genotype (p22phox CT + TT and RAGE GG). The frequency of p22phox CC and RAGE GT + TT (high-risk genotype) in group D was significantly higher than that in group N ($\chi^2 = 10.338$, $P = 0.0057$; 2 × 3). In multiple logistic regression analysis, HbA_{1c} level, triglyceride level, systolic blood pressure, and the combination of p22phox CC and RAGE GT + TT polymorphisms were shown to be independent variables ($P < 0.005$) (Table 4). These analyses revealed that the combination of these polymorphisms is a significant factor in the development of diabetic nephropathy.

CONCLUSIONS — NADPH oxidase is a critical enzyme for superoxide production in phagocytes, vascular smooth muscle cells, and mesangial cells (5). Recently, Guzik and colleagues (14,15) reported that superoxide production in human blood vessels from diabetic patients is mediated by upregulated NADPH oxidase activity through the PKC pathway. P22phox is an essential component of NADPH oxidase, and it has been reported that the C242T polymorphism is associated with vascular superoxide production in human blood vessels from CAD patients (5,6). The C242T polymorphism results in an amino acid polymorphism (His/Tyr) at residue 72, which is located in the putative heme-binding sites (16). Because the histidine residue is considered to be a candidate for the ligand of the heme prosthetic group of cytochrome *b*, it has been suggested that this polymorphism is directly associated with the function of p22phox (17). Inoue et al. (6) reported that the risk of CAD was lower in individuals carrying the 242T genotype in the Japanese population, whereas other investigations showed conflicting findings in patients with CAD and cerebrovascular disease (18–23). It has also been reported that this polymorphism is associated with significantly lower basal and NADPH-stimulated vascular superoxide production in human blood vessels from patients with atherosclerosis (14). Individuals with the 242T genotype might have lower oxidative stress as a result of lower O_2 production compared with individuals bearing the 242C genotype. In this study, we showed that patients with the 242T genotype have a lower risk of developing diabetic nephropathy. Our result was consistent with that of a previous

Table 3—*Distribution of p22phox C242T and RAGE G1704T genotypes in patient groups*

p22phox and RAGE	Group N	Group D	χ^2	*P*
CT + TT and GG	18 (17)	54 (5)		
CT + TT and GT + TT/CC and GG	81 (75)	53 (73)		
CC and GT + TT	9 (8)	16 (22)	10.338	0.0057

Data are *n* (%).

Table 4—*Multiple logistic regression analysis of candidate variables for risk factors for diabetic nephropathy*

Variables	OR (95% CI)	*P*
HbA_{1c}	1.54 (1.13–2.10)	0.0034
Triglyceride	1.01 (1.00–1.01)	0.0388
Systolic blood pressure	1.06 (1.03–1.09)	<0.0001
Retinopathy	1.78 (0.83–3.80)	0.067
p22phox CC and RAGE GT + TT	2.93 (1.34–6.41)	0.0073

Variables included in the model: onset age, disease duration, BMI at onset, HbA_{1c}, total cholesterol, triglyceride, retinopathy, p22phox and RAGE polymorphisms, and systolic blood pressure. $r^2 = 0.267$, $P < 0.0001$ for retinopathy (none = 0, retinopathy = 1) and for p22phox and RAGE polymorphisms (CT + TT and GG = 0, CT + TT and GT + TT/CC and GG = 1, CC and GT + TT = 2).

study of NADPH oxidase function (14). The T allele frequency in control subjects in our study was 0.11, which was similar to that in previous studies of Japanese populations, whereas it was ~33% of that in Caucasian populations (6,20,23).

AGEs by themselves have been shown to generate ROS and, conversely, the generation of AGEs is enhanced by oxidative stress (24). In vivo and in vitro studies indicate that AGEs play a role in the pathogenesis of diabetic nephropathy and the progression of renal failure (4). It has been reported that aminoguanidine and OPB-9195 are effective in preventing diabetic nephropathy (25,26). AGEs can bind to several binding sites, including RAGE. In RAGE-overexpressing mice, it has been demonstrated that the AGE-RAGE system is essential for the development of diabetic nephropathy (27). The AGE-RAGE interaction in mesangial and endothelial cells causes enhanced formation of oxygen radicals, with subsequent activation of NF-κB and release of proinflammatory cytokines and adhesion molecules, leading to collagen gene expression in mesangial cells (28). It has been reported that the A-374T polymorphism is associated with diabetic nephropathy in type 1 diabetic patients with poor metabolic control (4). The G1704T gene polymorphism has been reported to show a significant association with the complication of microvascular dermatoses and antioxidant status (8,9). Subjects bearing the RAGE 1704T polymorphism had significantly lower plasma levels of several antioxidants (total carotenoids, lutein, lycopene, and tocopherol) than those bearing RAGE 1704G, suggesting that this polymorphism may relate to oxidative stress and supporting our present results that patients with the 1704T genotype have a higher risk of developing diabetic nephropathy. The functional impact of the intron site is not clear, but possible mechanisms include alternative splicing, a change in mRNA stability, or a linkage of a nearby coding single nucleotide polymorphism.

We also examined the combination of the p22phox C242T and RAGE G1704T polymorphisms in relation to susceptibility to diabetic nephropathy. We showed that patients with the combination of p22phox CC and RAGE GT + TT genotypes had a significantly higher risk of diabetic nephropathy than those with the combination of p22phox CT + TT and RAGE GG genotypes. In this study, the RAGE G1704T gene polymorphism alone was weakly related to the susceptibility to diabetic nephropathy, but the combination of p22phox and RAGE gene polymorphisms was more significantly related to diabetic nephropathy. Both NADPH oxidase and RAGE are known to be expressed in mesangial cells, and recently, NADPH oxidase was reported to be a central target of RAGE; ROS generated by this mechanism could significantly impact cellular properties (10).

In conclusion, we propose that the combination of NADPH p22phox C242T and RAGE G1704T polymorphisms is associated with the development of diabetic nephropathy. It is obvious that microalbuminuria is the best documented predictor of the development of diabetic nephropathy (29). However, we propose that risk assessment based on gene polymorphisms, such as in this study, is also important. Further prospective studies are needed to clarify whether these polymorphisms can predict the development of diabetic nephropathy. Several studies have suggested that antioxidant treatment such as with ACE inhibitors (30), probucol (30), vitamin E (31), or α-lipoic acid (32) might be beneficial in preventing the development of diabetic nephropathy. It may be useful to identify the risk of the development of diabetic nephropathy and to select a medication such as an antioxidant.

References

1. Nishikawa T, Edelstein D, Brownlee M: The missing link: a single unifying mechanism for diabetic complications. *Kidney Int* 58:S26–S30, 2000
2. Pinizzotto M, Castillo E, Fiaux M, Temler E, Gaillard RC, Ruiz J: Paraoxonase2 polymorphisms are associated with nephropathy in type II diabetes. *Diabetologia* 44: 104–107, 2001
3. Nomiyama T, Tanaka Y, Piao L, Nagasaka K, Sakai K, Ogihara T, Nakajima K, Watada H, Kawamori R: The polymorphism of manganese superoxide dismutase is associated with diabetic nephropathy in Japanese type 2 diabetic patients. *J Hum Genet* 48:138–141, 2003
4. Pettersson-Fernholm K, Forsblom C, Hudson BI, Perola M, Grant PJ, Groop PH, Finn-Diane Study Group: The functional −374 T/A RAGE gene polymorphism is associated with proteinuria and cardiovascular disease in type 1 diabetic patients. *Diabetes* 52:891–894, 2003
5. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, Griedling KK: P22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. *J Biol Chem* 271:23317–23321, 1996
6. Inoue N, Kawashima S, Kanazawa K, Yamada S, Akita H, Yokoyama M: Polymorphism of the NADH/NADPH oxidase p22 phox gene in patients with coronary artery disease. *Circulation* 97:135–137, 1998
7. Yamamoto Y, Yamagishi S, Yonekura H, Doi T, Tsuji H, Kato I, Takasawa S, Okamoto H, Abedin J, Tanaka N, Sakurai S, Migita H, Unoki H, Wang H, Zenda T, Wu PS, Segawa Y, Higashide T, Kawasaki K, Yamamoto H: Roles of the AGE-RAGE system in vascular injury in diabetes. *Ann N Y Acad Sci* 902:163–170, 2000
8. Kankova K, Zahejsky J, Marova I, Muzik J, Kuhrova V, Blazkova M, Znojil V, Beranek M, Vacha J: Polymorphisms in the RAGE gene influence susceptibility to diabetes-associated microvascular dermatoses in NIDDM. *J Diabetes Complications* 15:185–192, 2001
9. Kankova K, Marova I, Zahejsky J, Muzik J, Stejskalova A, Znojil V, Vacha J: Polymorphisms 1704G/T and 2184A/G in the RAGE gene are associated with antioxidant status. *Metabolism* 50:1152–1160,

2001
10. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL: Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol* 280:E685–E694, 2001
11. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
12. Blin N, Stafford DW: A general method for isolation of high molecular weight DNA from eukaryocytes. *Nucleic Acid Res* 3:2303–2308, 1976
13. Whitcombe D, Brownie J, Gillard HL, McKechnie D, Theaker J, Newton CR, Little S: A homogeneous fluorescence assay for PCR amplicons: its application to real-time, single-tube genotyping. *Clin Chem* 44:918–923, 1998
14. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM: Functional effect of the C242T polymorphism in the NAD(P)H oxidase p22phox gene on vascular superoxide production in atherosclerosis. *Circulation* 102:1744–1747, 2000
15. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, Channon KM: Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxidase synthase. *Circulation* 105:1656–1662, 2002
16. Dinauer MC, Pierce EA, Bruns GA, Curnutte JT, Orkin SH: Human neutrophil cytochrome b light chain (p22-phox): gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. *J Clin Invest* 86:1729–1737, 1990
17. Wolin MS: How could a genetic variant of the p22 phox component of NAD(P)H oxidases contribute to the progression of coronary atherosclerosis? *Circ Res* 86:365–366, 2000
18. Gardemann A, Mages P, Katz N, Tillmanns H, Haberbosch W: The p22 phox A640G gene polymorphism but not the C242T gene variation is associated with coronary heart disease in younger individuals. *Atherosclerosis* 145:315–323, 1999
19. Cahilly C, Ballantyne CM, Lim DS, Gotto A, Marian AJ: A variant of p22(phox), involved in generation of reactive oxygen species in the vessel wall, is associated with progression of coronary atherosclerosis. *Circ Res* 86:391–395, 2000
20. Ito D, Murata M, Watanabe K, Yoshida T, Saito I, Tanahashi N, Fukuuchi Y: C242T polymorphism of NADPH oxidase p22 PHOX gene and ischemic cerebrovascular disease in the Japanese population. *Stroke* 31:936–939, 2000
21. Stanger O, Renner W, Khoschsorur G, Rigler B, Wascher TC: NADH/NADPH oxidase p22phox C242T polymorphism and lipid peroxidation in coronary artery disease. *Clin Physiol* 21:718–722, 2001
22. Cai H, Duarte N, Wilcken DE, Wang XL: NADH/NADPH oxidase p22 phox C242T polymorphism and coronary artery disease in the Australian population. *Eur J Clin Invest* 29:744–748, 1999
23. Hayaishi-Okano R, Yamasaki Y, Kajimoto Y, Sakamoto K, Ohtoshi K, Katakami N, Kawamori D, Miyatsuka T, Hatazaki M, Hazama Y, Hori M: Association of NAD(P)H oxidase p22 phox gene variation with advanced carotid atherosclerosis in Japanese type 2 diabetes. *Diabetes Care* 26:458–463, 2003
24. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, Shaw A: Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 267:14998–15004, 1992
25. Soulis T, Cooper ME, Vranes D, Bucala R, Jerums G: Effects of aminoguanidine in preventing experimental diabetic nephropathy are related to the duration of treatment. *Kidney Int* 50:627–634, 1996
26. Nakamura S, Makita Z, Ishikawa S, Yasumura K, Fujii W, Yanagisawa K, Kawata T, Koike T: Progression of nephropathy in spontaneous diabetic rats is prevented by OPB-9195, a novel inhibitor of advanced glycation. *Diabetes* 46:895–899, 1997
27. Yamamoto Y, Kato I, Doi T, Yonekura H, Ohashi S, Takeuchi M, Watanabe T, Yamagishi S, Sakurai S, Takasawa S, Okamoto H, Yamamoto H: Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. *J Clin Invest* 108:261–268, 2001
28. Heidland A, Sebekova K, Schinzel R: Advanced glycation end products and the progressive course of renal disease. *Am J Kidney Dis* 38:S100–S106, 2001
29. Parving HH, Chaturvedi N, Viberti G, Mogensen CE: Does microalbuminuria predict diabetic nephropathy? *Diabetes Care* 25:406–407, 2002
30. Nishimura M, Sasaki T, Ohishi A, Oishi M, Kono S, Totani Y, Kato Y, Noto Y, Misaki S, Higashi K, Shimada F, Wakasugi H, Inoue K, Hoshiyama Y, Yamada K: Angiotensin-converting enzyme inhibitors and probucol suppress the time-dependent increase in urinary type IV collagen excretion of type II diabetes mellitus patients with early diabetic nephropathy. *Clin Nephrol* 56:96–103, 2001
31. Bursell SE, King GL: Can protein kinase C inhibition and vitamin E prevent the development of diabetic vascular complications? *Diabetes Res Clin Pract* 45:169–182, 1999
32. Morcos M, Borcea V, Isermann B, Gehrke S, Ehret T, Henkels M, Schiekofer S, Hofmann M, Amiral J, Tritschler H, Ziegler R, Wahl P, Nawroth PP: Effect of alpha-lipoic acid on the progression of endothelial cell damage and albuminuria in patients with diabetes mellitus: an exploratory study. *Diabetes Res Clin Pract* 52:175–183, 2001