Adam Stefański[1], Mirosław Franków[1], Agnieszka Bińczak-Kuleta[2], Krzysztof Safranow[3], Piotr Molęda[1], Andrzej Ciechanowicz[2], Liliana Majkowska[1]

ORIGINAL

[1]Department of Diabetology and Internal Diseases, Pomeranian Medical University, Szczecin, Poland,
[2]Department of Laboratory Diagnostics and Molecular Medicine, Pomeranian Medical University, Szczecin, Poland,
[3]Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland

Association between genetic variants in the 3' untranslated region of the gene encoding type 2 tumor necrosis factor receptor (TNFRSF1B) and long-term diabetic complications in obese patients with type 2 diabetes

Abstract

Background. Tumor necrosis factor-alpha system seems to be involved in pathogenesis of chronic diabetic complications and genetic background may modulate its activity. The aim of the study was to evaluate a possible relationship between the polymorphism in the 3' untranslated region (3' UTR) of the TNFRSF1B gene and body weight, insulin resistance, insulin secretion and prevalence of diabetic complications in obese patients with type 2 diabetes.

Methods. One hundred and fifteen obese patients with at least 10-year history of type 2 diabetes were characterized clinically and the polymorphism in the 3' UTR of the TNFRSF1B gene was assessed by direct DNA sequencing. Five TNFRSF1B haplotypes were identified and differences between respective haplotype carriers and non-carriers were analyzed.

Results. No difference between the groups in variables describing body weight, insulin resistance and insulin secretion was found. Diabetic retinopathy was more frequent in patients with A2 haplotype and prevalence of myocardial infarction was higher in individuals with A1 haplotype as compared to respective non-carriers.

Conclusions. In obese patients with long-lasting type 2 diabetes some genetic variants in the 3' UTR of the TNFRSF1B gene are associated with prevalence of diabetic retinopathy and myocardial infarction. The results support hypothesis on a possible role of genetic heterogeneity in the TNFRSF1B gene in pathogenesis of chronic complications of diabetes.

key words: type 2 diabetes, diabetic complications, TNFRSF1B gene polymorphism

Introduction

Tumor necrosis factor-alpha (TNFα) is considered as one of the main cytokines implicated in inflammatory responses. Under chronic hyperglycemia endogenous TNFα production is accelerated in microvascular and neural tissues and through different mechanisms may lead to development of lesions characteristic for diabetic microangiopathy and polyneuropathy [1]. Several observations link inflammation and TNFα with development of insulin resistance, a crucial element in the pathogenesis of type 2 diabetes, but also with endothelial dysfunction, hypertension [2] and lipid abnormalities. Therefore it is possible that the TNFα system is directly or indirectly involved in development of chronic diabetic complications. Indeed, in many studies an association between the TNFα system and various microvascular and macrovascular complications was reported, both in type 1 and type 2 diabetes [3–6].

Most of the responses to TNFα are mediated by two membrane receptors, TNFR1 (p55) and TNFR2 (p75).

Address for correspondence: Adam Stefański, MD
Department of Diabetology and Internal Diseases
Pomeranian Medical University
ul. Arkońska 4, 71–455 Szczecin, Poland
tel: +48 91 431 62 41; fax: +48 91 431 62 43
e-mail: stefend@sci.pam.szczecin.pl

Diabetologia Doświadczalna i Kliniczna 2007, 7, 4, 192–198
Copyright © 2007 Via Medica, ISSN 1643–3165

After binding the cytokine to TNFR1 or TNFR2, a proteolytic cleavage of the extracellular parts of these receptors elicits the soluble forms, named sTNFR1 and sTNFR2 [7]. Kulseng et al. observed that in patients with type 1 diabetes serum levels of both soluble receptor types increased with severity of diabetic complications [8]. Unlike sTNFR1, sTNFR2 was shown to be increased in patients with peripheral vascular disease and in survivors of a myocardial infarction [9].

In order to explain a role of the TNFα system in pathogenesis of diabetic complications it is important to investigate factors potentially influencing its function. One of such factors is a genetic background. Several polymorphisms in the TNFα, TNF receptor 1 and TNF receptor 2 genes were described and reported to modulate the risk of diabetic retinopathy [10–12], nephropathy [13] and of coronary artery disease [14–17]. In the TNFRSF1B locus several mutational variants have been described. A microsatellite marker in intron 4 of the TNFRSF1B gene was reported to be associated with essential hypertension, hypercholesterolemia [2], coronary artery disease [16], and with susceptibility to familial combined hyperlipidemia [18]. In patients with type 2 diabetes genetic variation in or near TNFRSF1B locus has been suggested to predispose for clinical neuropathy, reduced glycosylated hemoglobin, and increased HDL-cholesterol [19]. However in another study in patients with type 2 diabetes none of variants in intron 4 of the TNFRSF1B gene predisposed to microvascular diabetic complications, although one of the variants was associated with elevated plasma HDL-cholesterol [6]. In the same study the finding of no association with diabetic complications was related also to a single nucleotide polymorphism at codon 196 (ATG > AGG, Met > Arg) in exon 6 of the TNFRSF1B gene [6].

Another polymorphism of the TNFRSF1B gene was described in the 3' untranslated region (UTR) [20]. It was shown that some variant alleles in this region were associated with obesity and insulin resistance in patients with type 2 diabetes [21]. Recently, Puga et al. [22] investigated whether the polymorphism in the 3' UTR of the TNFRSF1B gene could affect expression of the gene using reporter constructs in lymphoid Jurkat T cells. Results of the study suggest that an increased rate of TNFR2 mRNA degradation, protecting cells from unrestrained TNFα effects, is weakened in carriers of one of haplotypes, which may result in increased levels of TNFR2 expression and enhanced effects of TNFα in subjects carrying the haplotype. The findings may indicate that some variants in the 3' UTR of the TNFRSF1B gene indeed affect the TNFα system activity and may influence the development of chronic diabetic complications.

Since analysis of more homogenous groups of patients in a clinical trial searching for genotype/pheno-type associations should improve its sensitivity and pathogenesis of carbohydrate metabolism abnormalities in type 2 diabetes may be different in lean and in obese individuals, we decided to study only obese diabetic patients.

The aim of the present study was to evaluate a possible relationship between the polymorphism in the 3' untranslated region of the gene for TNFRSF1B and body weight, insulin resistance, insulin secretion and the presence of chronic diabetes complications in obese patients with long-lasting type 2 diabetes.

Material and methods

One hundred and fifteen consecutive patients (54 men and 61 women) who had a body mass index (BMI) $\geq$ 30 kg/m^2 and a documented 10-years or longer history of type 2 diabetes were recruited for the study from a Regional Diabetic Center. The study protocol was approved by the institutional Ethics Committee. All subjects gave informed consent before their enrollment into the study. A detailed interview with special emphasis on a history of diabetes and medication used was conducted with all the patients. A presence of diabetic complications was evaluated on a basis of funduscopy (performed by ophthalmologist), foot examination (palpation of pulse on peripheral arteries and testing sense abnormalities), electrocardiography, microalbuminuria (RIA, ImmunoTech, Czech Republic) and serum creatinine determination (Jaffe method). Diabetic nephropathy was diagnosed in patients with microalbuminuria over 30 mg/day only when a presence of diabetic retinopathy had been confirmed. Diabetic neuropathy was diagnosed on the basis of the interview and finding of vibration or pain sense abnormalities on physical examination. The diagnosis of ischemic heart disease (IHD) required a history of one of the following: 1) a history of myocardial infarction; 2) coronary angioplasty; 3) bypass surgery; 4) presence of coronary lesions on angiography or 5) presence of typical symptoms of angina pectoris on interview together with typical changes on electrocardiography. Brain stroke was diagnosed on the basis of data in medical records of a patient, including CT scan of brain results confirming the diagnosis. All the patients underwent a physical examination with evaluation of body mass index (BMI) and waist/hip ratio (WHR). Measurements of biochemical parameters included glycated hemoglobin A_{1c} in blood (HbA_{1c}, measured by HPLC, Bio-Rad Laboratories Inc, California, USA), serum glucose (enzymatic method, Cormay, Poland), C-peptide, soluble TNFR2 (RIA, BioSource Europe, Belgium), triglycerides, total cholesterol and its fractions (enzymatic method, Cobas Integra, Roche Diagnostics,

Table 1. Characterization of variants in 3'UTR (exon 10) of the TNFRSF1B gene and frequency of these variants in the study group
Table 1a. Frequency of TNFRSF1B haplotypes in the study group

Nucleotides	Sequence	Haplotype*	Frequency
1663–1668–1690	G T T	A1	0.47
	A T C	A2	0.24
	A G T	A3	0.04
	A T T	A4	0.13
	G T C	A5	0.12

Table 1b. TNFRSF1B diplotypes in the study group

Diplotype	Number of subjects	Diplotype	Number of subjects	Diplotype	Number of subjects
A1/A1	23	A2/A2	3	A3/A4	0
A1/A2	29	A2/A3	2	A3/A5	1
A1/A3	7	A2/A4	6	A4/A4	5
A1/A4	13	A2/A5	12	A4/A5	0
A1/A5	13	A3/A3	0	A5/A5	1

*According to Puga et al. [22]

Switzerland). In all the patients β-cell function was estimated by measurement of serum C peptide basal levels and 6 minutes after intravenous stimulation with 1 mg of glucagon (GlucaGen HypoKit, Novo Nordisk, Denmark). On the day of the glucagon test the patients were advised not to take their medications in the morning and to present on fasting between 7:00 and 9:00 AM for blood taking. The patients were on their usual diet with no special recommendations concerning physical activity. To characterize β-cell function more precisely a HOmeostatic Model Assessment 2 (HOMA2) was applied (Holman et al., 2004). C-peptide and glucose concentrations measured during glucagon stimulatory test were used for assessment of parameters describing insulin secretion (HOMA2-%B) and insulin sensitivity (HOMA2--%S). To calculate all the parameters we used C-peptide concentration as a surrogate of insulin concentration, since a part of the patients was treated with insulin injections.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes with nonenzymatic and nonorganic method with use of 2% Triton X-100 solution [23]. DNA was amplified with specific flanking primers for the 3' UTR of TNFR2 (TNFRSF1B gene) as described previously by Fernandez-Real et al. [21]. The forward primer was as follows: 5'-AGGACTCTGAGGCTCTTTCT-3'. The reverse primer was as follows: 5'-TCACAGAGAGTCAGG-GACTT-3'. For genotyping of three single nucleotide polymorphisms at positions 1663, 1668 and 1690 (593, 598 and 620 according to the reference 21) from transcript start (rs1061624, rs5030792 and rs3397, respectively) a method of direct DNA sequencing was used.

Statistical analysis

We identified five haplotypes of the TNFRSF1B gene (A1–A5) (Table 1) [22] and for a further evaluation we divided our study group according to the type of the haplotype (Table 1). The software STATISTICA version 7.1 (StatSoft Inc., Tulsa, OK, USA) was used for database management and statistical analysis. Hardy-Weinberg equilibrium was assessed by χ^2 analysis. The relationship of genotype with various parameters was tested by presence or absence of the analyzed haplotype (i.e. A1 versus non-A1, A2 versus non-A2, etc). Since distribution of most of analyzed continuous variables differed significantly from normal distribution (Shapiro--Wilk's test) non-parametric Mann-Whitney test was used to check for significance of differences between haplotype groups. The differences in nominal variables between respective haplotype carriers and non-carriers were analyzed with Fisher exact test. Univariate and multivariate (adjusted for age, gender, HbA_{1c} and diabetes duration) logistic regression models were used to study the associations between TNFRSF1B haplotypes and clinical parameters. P-value of < 0.05 was considered as statistically significant.

Results

The distribution of genotypes was consistent with Hardy-Weinberg equilibrium for all three loci (P = 0.25, 1.0 and 0.69 for polymorphisms at position 1663, 1668 and 1690, respectively). No significant difference between the analyzed groups in such variables as age, gender, BMI, WHR, diabetes duration, creatinine, triglycerides, total cholesterol and LDL-cholesterol was found (Table 2). However, unlike for other haplotypes, A2-allele carriers were more often (P = 0.052) treated with insulin in comparison to non-A2 carriers (Table 2 and Table 3). Also in univariate and multivariate logistic regression models the higher number of A2 alleles was associated with higher probability of insulin treatment (Table 4). The analysis of results of the intravenous glucagon stimulatory test did not show any significant difference between the analyzed groups in serum glucose and C-peptide before and after stimulation as well as HOMA2%-B and HOMA2%-S (Table 2).

The evaluation of long-term complications of diabetes did not reveal a difference in prevalence of diabetic nephropathy and neuropathy between the analyzed groups. However, diabetic retinopathy (but not proliferative retinopathy) was significantly more frequent in patients with the haplotype A2 as compared with non-A2 carriers (Table 3). In univariate and multivariate logistic regression models the number of A2 alleles was also significantly associated with presence of retinopathy (Table 4).

Diabetic macroangiopathic complications like brain stroke and peripheral artery disease occurred with similar frequency in all the groups. Also prevalence of coronary heart disease did not differ between carriers and non-carriers of all analyzed haplotypes. However, prevalence of past myocardial infarction was significantly higher (P = 0.035) in haplotype A1 carriers (25%: 21 out of 84 individuals — no data available for 1 patient) compared to non-A1 individuals (7%: 2 out of 30 individuals). In univariate logistic regression model odds ratio for increased prevalence of past myocardial infarction in individuals carrying A1 allele was significant (OR: 4.67; 95% CI: 1.01–21.63; P = 0.047). In multivariate logistic regression analysis, after adjustment for patient's age, gender, HbA_{1c} and diabetes duration, the association was of borderline significance (OR: 4.35; 95% CI: 0.91–20.75; P = 0.062).

Discussion

The results of our study demonstrate for the first time that polymorphisms in the 3' UTR of the TNFRSF1B gene are associated with development of chronic complications of diabetes. We found that the presence of the haplotype A2 may predispose to development of diabetic retinopathy. The fact that A2-haplotype carriers were significantly more often treated with insulin than non-A2 carriers suggests more advanced insulin secretion failure or more problems with metabolic control. The duration of diabetes and glycemic control are well established risk factors for the development of diabetic retinopathy [24, 25]. However we were not able to demonstrate any difference in HbA_{1c}, in fasting serum glucose and in parameters describing insulin secretion and insulin resistance between the carriers and non-carriers of the A2 haplotype. Also duration of diabetes was similar in both groups. Another possibility is that the predisposition to development of diabetic retinopathy is related mainly to an inflammatory process with changed activity of the TNFα system. Joussen et al. [26] demonstrated that nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNFα suppression. Since soluble TNFR2 has been proposed as the best predictor of the TNFα system activity [27] and in patients with type 1 diabetes higher serum levels of sTNFR1 and sTNFR2 were observed in individuals with proliferative retinopathy than in those without retinopathy [5], we measured serum sTNFR2 levels in patients participating in our study. We found no difference in serum sTNFR2 concentrations between carriers and non-carriers of the A2 haplotype. However only in a few patients proliferative retinopathy was present. It is possible that in our relatively small study group severity of retinal changes in patients with retinopathy was not sufficient to enable detection of differences in serum sTNFR2 levels. In our study we did not find an association between any of analyzed haplotypes and prevalence of proliferative diabetic retinopathy (Table 3), perhaps due to too small number of individuals with this complication.

Another interesting finding is more frequent incidence of myocardial infarction in A1 haplotype carriers compared to non-A1 individuals, suggesting that the presence of this haplotype of the TNFRSF1B gene may increase a risk of this complication. There are observations suggesting a role of changed TNFα system activity in the pathogenesis of myocardial infarction. In rats gene transfer of the soluble TNFα receptor 1 to the heart reduced the TNFα bioactivity in the cardiomyocytes and thereby protected the myocardium from acute myocardial infarction *in vivo* [28]. In human plasma TNFα levels were shown to be elevated early on the course of acute myocardial infarction and treatment with the anti-TNFα monoclonal antibody decreased area of necrosis, indicating that TNFα release contributed to myocardial injury [29]. Genetic reports evaluating relationship between various polymorphisms in the TNFRSF1B gene and

Table 2. Basal clinical and biochemical characteristics of patients with various TNFRSF1B haplotypes

Variables	Haplotypes					
	A1 (n = 85)	**Non-A1 (n = 30)**	**P value***	**A2(n = 52)**	**Non-A2 (n = 63)**	**P value****
Age (years)	64.7 ± 7.8	62.4 ± 8.8	0.23	63.5 ± 7.8	64.6 ± 8.4	0.46
Gender (% of males)	47%	43%	0.83	50%	43%	0.46
BMI [kg/m^2]	33.7 ± 3.5	34.7 ± 3.5	0.090	33.7 ± 3.3	34.1 ± 3.7	0.62
Body weight change during disease [kg]	–0.9 ± 15.0	–1.7 ± 14.2	0.61	–0.9 ± 13.6	–1.2 ± 15.7	0.79
WHR	0.98 ± 0.06	0.97 ± 0.08	0.49	0.98 ± 0.06	0.97 ± 0.07	0.29
Diabetes duration (years)	16.5 ± 6.2	16.4 ± 5.5	0.89	16.5 ± 5.9	16.5 ± 6.1	0.98
Patients treated with insulin (%)	60%	70%	0.39	73%	54%	0.052
Duration of insulin treatment (years) (n = 71)	11.1 ± 5.7	10.2 ± 6.3	0.41	11.7 ± 5.7	9.9 ± 6.3	0.23
HbA_{1c} (%)	7.9 ± 1.4	7.9 ± 1.8	0.61	7.9 ± 1.6	7.9 ± 1.4	0.91
Total cholesterol [mg/dL]	221.2 ± 51.8	225.2 ± 51.6	0.72	218.3 ± 50.5	225.6 ± 52.7	0.60
LDL-cholesterol [mg/dL]	135.8 ± 38.8	142 ± 47.5	0.76	135 ± 43.9	139.4 ± 39	0.32
HDL-cholesterol [mg/dL]	51.1 ± 12.6	52.9 ± 14.2	0.47	51.6 ± 15.4	51.6 ± 10.7	0.46
Triglycerides [mg/dL]	177.5 ± 103.6	175.2 ± 69.5	0.56	174.6 ± 74.6	178.9 ± 110.8	0.57
Creatinine [mg/dL]	1.01 ± 0.47	0.95 ± 0.28	0.38	0.96 ± 0.28	1.02 ± 0.53	0.55
sTNFR2 [ng/mL]	8.22 ± 3.3	8.48 ± 3.54	0.82	8.59 ± 3.62	8.04 ± 3.12	0.46
HOMA2%-B	36.1 ± 35	32.1 ± 25.2	0.93	38.1 ± 39.7	32.5 ± 25.5	0.68
HOMA2%-S	209 ± 161	256 ± 236	0.59	237 ± 205	209 ± 164	0.65

*A1 carriers *vs*. non-carriers; **A2 carriers *vs*. non-carriers; Mann-Whitney test was used for quantitative variables and Fisher exact test for qualitative variable

Table 3. Prevalence of diabetic retinopathy and insulin treatment with regard to A2 haplotype in 3'UTR (exon 10) of the TNFRSF1B gene

Group	n	Combination of haplotypes			P value**
		A2/A2	A2/non-A2	Non-A2/non-A2	
Retinopathy*	53	2 (3.8%)	26 (49.0%)	25 (47.2%)	0.031
No retinopathy*	51	0 (0%)	16 (31.4%)	35 (68.6%)	
Proliferative retinopathy*	9	0 (0%)	5 (56%)	4 (44%)	0.49
No proliferative retinopathy*	95	2 (2%)	37 (39%)	56 (59%)	
Insulin treatment	72	3 (4.2%)	35 (48.6%)	34 (47.2%)	0.052
No insulin treatment	43	0 (0%)	14 (32.6%)	29 (67.4%)	

*Ophthalmological evaluation done in 104 patients out of 115; **P values for the difference between carriers and non-carriers of A2 haplotype (Fisher exact test)

Table 4. Univariate and multivariate logistic regression models predicting odds ratios for presence of retinopathy and for insulin treatment in relation to the number of TNFRSF1B A2 alleles

	Diabetic retinopathy	
Logistic regression model	Number of TNFRSF1B A2 alleles*	
	OR (95% CI)	P
Univariate	2.52 (1.16–5.45)	0.018
Multivariate**	2.90 (1.17–7.17)	0.020
	Insulin treatment	
Logistic regression model	**Number of TNFRSF1B A2 alleles***	
	OR (95% CI)	**P**
Univariate	2.38 (1.12–5.06)	0.023
Multivariate**	2.54 (1.06–6.06)	0.033

*The number of A2 alleles (0, 1 or 2) was the independent variable, therefore odds ratios express the increased prevalence of retinopathy or frequency of insulin treatment associated with each copy of A2 allele; **adjusted for patient's age, gender, HbA_{1c} and diabetes duration

coronary artery disease have yielded inconsistent results. Sankar et al. [17] found an association between a polymorphism at position 196 in exon 6 of TNFRSF1B gene and development of coronary artery disease in an Indian population, whereas in a study in a British cohort the same polymorphism was not associated with the disease [30]. In another group of non-diabetic individuals Benjafield et al. [16] observed an association of a microsatellite marker in intron 4 of the TNFRSF1B gene (TNFRSF1B) with incidence of the coronary artery disease and suggested that genetic variation in or near the TNFRSF1B locus may predispose to the disease. However in a study in patients with type 2 diabetes the only complication associated with TNFRSF1B was clinical neuropathy, but not retinopathy or myocardial infarction [19].

On the basis of available data we cannot speculate on possible mechanisms linking genetic variants in the 3' UTR of TNFRSF1B gene with different prevalence of diabetic retinopathy or myocardial infarction. Nevertheless results of our study support hypothesis on a possible role of genetic heterogeneity in TNFRSF1B gene in the pathogenesis of chronic complications of diabetes.

Acknowledgments

The study was supported by the Polish Scientific Research Committee (grant No 6P05B05020).

References

1. Satoh J, Yagihashi S, Toyota T. The possible role of tumor necrosis factor-alpha in diabetic polyneuropathy. Exp Diabesity Res 2003; 4: 65–71.
2. Glenn CL, Wang WYS, Benjafield AV, Morris BJ. Linkage and association of tumor necrosis factor receptor 2 locus with hypertension, hypercholesterolemia and plasma shed receptor. Hum Mol Genet 2000; 13: 1943–1949.
3. Gonzalez-Clemente JM, Mauricio D, Richart C. et al. Diabetic neuropathy is associated with activation of the TNF-alpha system in subjects with type 1 diabetes mellitus. Clin Endocrinol (Oxf) 2005; 63: 525–529.
4. Zoppini G, Faccini G, Muggeo M, Zenari L, Falezza G, Targher G. Elevated plasma levels of soluble receptors of TNF-alpha

and their association with smoking and microvascular complications in young adults with type 1 diabetes. J Clin Endocrinol Metab 2001; 86: 3805–3808.
5. Limb GA, Soomro H, Janikoun S, Hollifield RD, Shilling J. Evidence for control of tumour necrosis factor-alpha (TNF-alpha) activity by TNF receptors in patients with proliferative diabetic retinopathy. Clin Exp Immunol 1999; 115: 409–414.
6. Vendrell J, Broch M, Fernandez-Real JM. et al. Tumour necrosis factor receptors (TNFRs) in type 2 diabetes. Analysis of soluble plasma fractions and genetic variations of TNFR2 gene in a case-control study. Diabet Med 2005; 22: 387–392.
7. Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med 1992; 175: 323–329.
8. Kulseng B, Vatten L, Espevik T. Soluble tumor necrosis factor receptors in sera from patients with insulin-dependent diabetes mellitus: relations to duration and complications of disease. Acta Diabetol 1999; 36: 99–105.
9. Blann AD, McCollum CN. Increased levels of soluble tumor necrosis factor receptors in atherosclerosis: no clear relationship with levels of tumor necrosis factor. Inflammation 1998; 22: 483–491.
10. Kumaramanickavel G, Sripriya S, Vellanki RN. et al. Tumor necrosis factor allelic polymorphism with diabetic retinopathy in India. Diabetes Res Clin Pract 2001; 54: 89–94.
11. Hawrami K, Hitman GA, Rema M. et al. An association in non-insulin-dependent diabetes mellitus subjects between susceptibility to retinopathy and tumor necrosis factor polymorphism. Hum Immunol 1996; 46: 49–54.
12. Kankova K, Muzik J, Karaskova J. et al. Duration of non-insulin-dependent diabetes mellitus and the TNF-α NcoI genotype as predictive factors in proliferative diabetic retinopathy. Ophthalmologica 2001; 215: 294–298.
13. Wang Y, Ng MC, So WY. et al. Association between tumour necrosis factor-alpha G-308A polymorphism and risk of nephropathy in obese Chinese type 2 diabetic patients. Nephrol Dial Transplant 2005; 20: 2733–2738.
14. Vendrell J, Fernandez-Real JM, Gutierrez C. et al. A polymorphism in the promoter of the tumor necrosis factor-alpha gene (–308) is associated with coronary heart disease in type 2 diabetic patients. Atherosclerosis 2003; 167: 257–264.
15. Dedoussis GV, Panagiotakos DB, Vidra NV. et al. Association between TNF-alpha -308G > A polymorphism and the development of acute coronary syndromes in Greek subjects: the CARDIO2000-GENE Study. Genet Med 2005; 7: 411–416.
16. Benjafield AV, Wang XL, Morris BJ. Tumor necrosis factor receptor 2 gene (TNFRSF1B) in genetic basis of coronary artery disease. J Mol Med 2001; 79: 109–115.
17. Sankar VH, Girisha KM, Gilmour A. et al. TNFR2 gene polymorphism in coronary artery disease. Indian J Med Sci 2005; 59: 104–108.
18. Geurts JM, Janssen RG, van Greevenbroek MM. et al. Identification of TNFRSF1B as a novel modifier gene in familial combined hyperlipidaemia. Hum Mol Genet 2000; 9: 2067–2074.
19. Benjafield AV, Glenn CL, Wang XL, Colagiuri S, Morris BJ. TNFRSF1B in genetic predisposition to clinical neuropathy and effect on HDL cholesterol and glycosylated hemoglobin in type 2 diabetes. Diabetes Care 2001; 24: 753–757.
20. Pantelidis P, Lympany PA, Foley PJ, Fanning GC, Welsh KI, du Bois RM. Polymorphic analysis of the high-affinity tumor necrosis factor receptor 2. Tissue Antigens 1999; 54: 585–591.
21. Fernandez-Real JM, Vendrell J, Ricart W. et al. Polymorphism of the tumor necrosis factor-α receptor 2 gene is associated with obesity, leptin levels, and insulin resistance in young subjects and diet-treated type 2 diabetic patients. Diabetes Care 2000; 23: 831–837.
22. Puga I, Lainez B, Fernandez-Real JM. et al. A polymorphism in the 3' untranslated region of the gene for tumor necrosis factor receptor 2 modulates reporter gene expression. Endocrinology 2005; 146: 2210–2220.
23. Lahiri DK, Bye S, Nurnberger Jr JI, Hodes ME, Crisp M. A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. J Biochem Biophys Methods 1992; 25: 193–205.
24. Cahill G, Etzweiler L, Freinkel N. Control and diabetes. N Engl J Med 1976; 294: 1004–1005.
25. The Diabetes Control and Complications Trial Research Group. The effect of intensive insulin treatment of diabetes on the development and progression of long term complications in insulin dependent diabetes mellitus. N Engl J Med 1993; 329: 977–986.
26. Joussen AM, Poulaki V, Mitsiades N. et al. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. FASEB J 2002; 16: 438–440.
27. Hotamisligil GS, Arner P, Atkinson RL, Spiegelman BM. Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance. Diabetes 1997; 46: 451–455.
28. Sugano M, Koyanagi M, Tsuchida K, Hata T, Makino N. *In vivo* gene transfer of soluble TNF-alpha receptor 1 alleviates myocardial infarction. FASEB J 2002; 16: 1421–1422.
29. Li D, Zhao L, Liu M. et al. Kinetic of tumor necrosis factor-α in plasma and the cardioprotective effect of a monoclonal antibody to tumor necrosis factor-α in acute myocardial infarction. Am Heart J 1999; 137: 1145–1152.
30. Allen RA, Lee EM, Roberts DH, Park BK, Pirmohamed M. Polymorphisms in the TNF-alpha and TNF-receptor genes in patients with coronary artery disease. Eur J Clin Invest 2001; 31: 843–851.