



Different Risk Indicators of Diabetic Nephropathy in Transforming Growth Factor-beta1 T869C CC/CT Genotype and TT Genotype

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Abstract

Background: Transforming growth factor-beta 1 (TGF- β 1) T869C (rs1800470, the same below) gene polymorphism is notably relative with the development of Diabetic Nephropathy (DN), and CC/CT genotype diabetic have higher frequency of than TT genotype diabetic. To find out individual risk factors in the two genotypes especially in susceptible genotype could provide more efficient and targeted prevention.

Methods: This was a prospective cohort study. A total of 251 type 2 diabetes mellitus (T2DM) patients [53.4% male, 56(52-67) years] were enrolled in this cohort study. Multiple concerned factors were collected and the relationship of these risk factors and development of DN were evaluated by Cox regression analysis. Hazard ratios of development of DN were calculated by Kaplan-Meier curves and the Cox proportional hazards model for CC/CT genotype versus TT genotype patients.

Results: TGF- β 1 T869C gene polymorphism was an independent predictor of DN in T2DM patients (HR, 2.08; 95%CI, 1.18-3.66; $P=0.012$). Hyperlipemia (HR, 1.91; 95%CI, 1.19-3.08; $P=0.007$), age (HR, 0.95; 95%CI, 0.93-0.98; $P=0.001$) and smoking status (HR, 2.36; 95%CI, 1.07-5.21; $P=0.033$) were risk indicators of the development of DN in CC/CT genotype patients. HbA1c (HR, 2.8; 95%CI, 1.07-7.30; $P=0.036$), hypertension (HR, 7.46; 95%CI, 1.38-40.29; $P=0.02$), and hyperlipemia (HR, 12.33; 95%CI, 1.05-145.39; $P=0.046$) were risk indicators for the development of DN in TT genotype patients.

Conclusion: TGF- β 1 T869C gene polymorphism was an independent predictor of DN for T2DM patients and CC/CT genotype had higher susceptibility to DN. CC/CT genotype and TT genotype patients had different risk indicators of DN.

Keywords: TGF- β 1, Diabetic nephropathy, T869C, Risk, Type 2 diabetes mellitus

Introduction

Diabetic Nephropathy (DN) is one of the most conventional microvascular complications of diabetes mellitus. Approximately 30%-40% of all patients with diabetes develop DN and it is the primary leading cause of end stage renal disease (ESRD) (1). Meanwhile, the ESRD is the main cause of death in type 2 diabetes mellitus (T2DM). Therefore, it is important to prevent diabetes developing into DN (2, 3).

Many factors, such as hypertension, hyperglycemia, the accumulation of advanced glycation products, dyslipidemia, and albuminuria/proteinuria, increase the risk of DN. Besides, diabetic patients may have increased susceptibility to DN due to genetic factors (4-6), such as transforming growth factor- β 1 (TGF- β 1) T869C (Leu 10 Pro) gene polymorphism. Transforming growth factor-beta (TGF- β) is a multifunctional regulator that modulates cell proliferation, differentiation, apoptosis,

adhesion and migration of various cell types and induces the production of extracellular matrix proteins (7). "It is synthesized by many renal cell types and exerts its biological functions through a variety of signalling pathways, including the Smad and MAPK pathways" (8). "In renal diseases, TGF- β is upregulated and induces renal cells to produce extracellular matrix proteins leading to glomerulosclerosis as well as tubulointerstitial fibrosis, which ultimately result in renal dysfunction" (9). T869C gene polymorphism was notable different between patients with DN and diabetic patients without DN, and the DN group had higher frequency of CC/CT genotype than the diabetes without DN group (5). Similar results were also found in other studies (10, 11). These conclusions were in line with our previous findings (12). T869C gene polymorphism has not yet been studied in prospective cohort study in T2DM patients. We thereby conducted a study to confirm whether T869C gene polymorphism contributes to the established predictor of DN, and secondly, to find out different risk factors to provide a better and possibly different treatment for susceptible populations (T869C CC/CT genotype) and non-susceptible populations (T869C TT genotype).

Materials and Methods

Study patients

This was a prospective, observational cohort study. All of the patients admitted met the diagnostic criteria of DM proposed by WHO in 1999, the Chinese Medical Association, and the diagnostic criteria of T2DM in "China Guideline for Diabetes (Trial Version)"(13), and DN was defined in accordance with the internationally recognized Mogensen staging criteria (14). All of the cases were recruited from January 2010 to May 2011, diagnosed as T2DM. Patients were followed every half year until May 2013. All of the patients were recruited in the Hangzhou Red Cross Hospital, with a DM duration >4 yr and no limit of sex and age. Individuals with a history of malignancy, receiving a kidney transplant, cerebral vascular accidents, or myocardial infarction were eliminated from this study. A total of 251 patients were enrolled finally.

Informed consent was obtained from all the patients before enrollment in the study. This study was performed and approved by the institutional Ethics Committee of our hospital.

Data Collection and laboratory measurements

The following demographic and clinical data were collected for each patient: age, gender, height, weight, primary renal disease, duration of DM, smoking status, drinking status, glycosylated hemoglobin (HbA1c) level, and comorbid conditions including hypertension, hyperlipemia. Fasting blood samples were checked for glucose, hemoglobin (HbA1c), and lipids. Arterial blood pressure was measured using a standard sphygmomanometer and hypertension was recorded if the patient was taking antihypertensive drugs or had two separated measured blood pressures >140/90 mmHg. Total cholesterol and low-density lipoprotein cholesterol (LDL-C) were measured by enzymatic method. Hyperlipidemic subjects had total cholesterol \geq 200 mg/dL and LDL-C \geq 100 mg/dL. HbA1c levels were measured by high-performance liquid chromatography.

We divided patients into CC/CT and TT group according to TGF- β 1 T869C genotype. For all participants, TGF- β 1 T869C genotype were identified, and the experimental method were as same as previously study (12). For all the subjects, 3 ml of venous blood were collected to take DNA samples using a blood DNA extraction kit (TaKaNa Co., Japan). PCR-RFLP was used to detect transforming growth factor (TGF)- β 1 (T869C) gene polymorphisms, PCR amplification to amplify genes, and Primer 5.0 software to design the primers: TGF- β 1 gene T869C upstream primer 5'-TTCCCTCGAGGCCCTCCTA and downstream primer 5'-GCCGCAGCTTGGACAGGATC. The synthesis was conducted by Sangon Biotech (Shanghai) Co., Ltd.

Statistical Analysis

Variables are presented in this study as median (interquartile range), or number (proportion) where appropriate. Differences in variables between the CC/CT and TT group were tested using the non-parametric Mann-Whitney test or the

chi-square test where appropriate. Spearman rank correlation matrix was created to evaluate the relationships among clinical variables. Cox regression analysis was used to evaluate the prognostic risks of DN. Two different approaches were applied: 1) univariate Cox regression analysis was selected from the following variables: age, gender, smoking status, drinking status, HbA1C level, hypertension, and hyperlipemia for the development of DN, and 2) the multivariate Cox regression analysis model using covariates by an entry selection procedure. Statistical significance was assumed for $P < 0.05$. Data were analyzed using SPSS 19.0 (Chicago, IL, USA).

Results

Patient characteristics at baseline

Baseline characteristics of the patients, when divided into groups according to TGF- β 1 T869C genotype, can be seen in Table 1. Median (interquartile range) age at baseline was 56 (52-67) yr. Diabetes duration at baseline was 9 (7-11) yr. Compared with TT genotype patients, CC/CT genotype patients had longer duration of diabetes [(9 (7-11) vs. 9 (5-11); $P=0.008$)], more frequently hypertension (76.0% vs. 33.3%; $P<0.001$), and smoking (79.8% vs. 19.4%; $P<0.001$). Successfully follow up were defined as development of DN or year 2013 without DN.

Risk indicators of DN in CC/CT or TT genotype

During follow-up to 2013, 11 patients and 87 patients had developed DN in TT genotype group and

CC/CT genotype group, respectively. DN incidence rates were 55.06% higher in the CC/CT genotype group than that of 11.83% in the TT genotype group ($P < 0.001$). Obviously, the cumulative hazard of developing DN was significantly higher in CC/CT genotype group than that in TT genotype group (HR, 3.27; 95% CI, 1.99-5.37; $P < 0.001$) (Fig. 1).

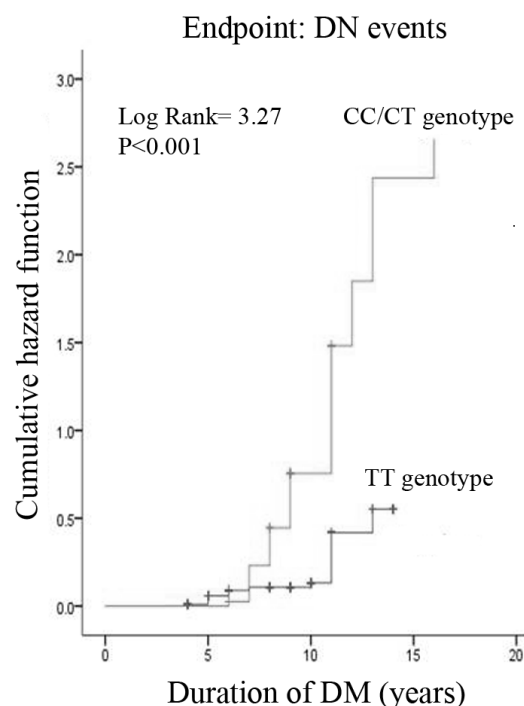


Fig. 1: Cumulative prevalence of DN events according to T869C polymorphism

Table 1: Baseline characteristics of 251 Diabetes mellitus patients

Characteristics	Total	CC/CTgenotype	TTgenotype	Pvalue
All, n(%)	251(100)	158 (62.9)	93 (37.1)	
Age (yr)	56 (52-67)	55 (49-67)	56 (54-67)	0.545
Male, n(%)	134 (53.4)	86 (54.4)	48 (51.6)	0.97
Smoking, n(%)	144 (57.4)	126 (79.8)	18 (19.4)	<0.001
Drinking, n(%)	138 (55)	94 (59.5)	44 (47.3)	0.354
HbA1c (%)	6.9 (6.3-7.4)	6.9 (6.3-7.4)	7.0 (6.4-7.5)	0.462
Hypertension, n(%)	151(60.2)	120 (76.0)	31(33.3)	<0.001
hyperlipemia, n(%)	120 (47.8)	76 (48.1)	44 (47.3)	0.547
Diabetes duration (yr)	9 (7-11)	9 (7-11)	9 (5-11)	0.008

Data are median (25th, 75th percentiles), or percentage.

The predictors of DN for the CC/CT genotype group are summarized in Table 2. In a univariate analysis, age, HbA1c, and smoking status were associated with risk of DN. However, in the multivariate model after adjusting, hyperlipemia (HR, 1.91; 95%CI, 1.19-3.08; $P=0.007$) took the place of HbA1c, along with age (HR, 0.95; 95%CI, 0.93-0.98; $P=0.001$) and smoking status (HR, 2.36; 95%CI, 1.07-5.21; $P=0.033$) became predictors of DN. In addition, hypertension another traditional risk indicators of DN, was not significant, singly or in combination.

Table 2: Univariate and multivariate Cox regression analysis for the risk indicators of DN in 158 Diabetes mellitus patients with CC/CT genotype

	Hazard ratio (95%CI)	P value
univariate Cox regression analysis		
Age (yr)	0.95 (0.93-0.97)	<0.001
Male	1.38 (0.96-1.97)	0.08
HbA1c	1.15 (1.01-1.318)	0.036
Smoking	2.13 (1.11-4.09)	0.023
Drinking	0.93 (0.65-1.32)	0.667
Hypertension	1.05(0.64-1.74)	0.851
Hyperlipemia	1.29(0.90-1.87)	0.167
multivariate Cox regression analysis		
Age (yr)	0.95(0.93-0.98)	<0.001
Smoking	2.36(1.07-5.21)	0.033
Hyperlipemia	1.91(1.19-3.08)	0.007

Table 3: Univariate and multivariate Cox regression analysis for the risk indicators of DN in 93 Diabetes mellitus patients with TT genotype

	Hazard ratio (95%CI)	P value
univariate Cox regression analysis		
Age (yr)	1.08 (1.01-1.15)	0.03
Male	0.77 (0.29-2.04)	0.597
HbA1c	1.99 (1.22-3.24)	0.006
Smoking	3.56 (1.35-9.43)	0.011
Drinking	0.34 (0.11-1.03)	0.057
Hypertension	13.93 (3.12-62.20)	0.001
Hyperlipemia	21.19 (2.70-166.35)	0.004
multivariate Cox regression analysis		
HBA1C	2.80 (1.07-7.30)	0.036
Hypertension	7.46 (1.38-40.29)	0.02
Hyperlipemia	12.33 (1.05-145.39)	0.046

The predictors of DN for the TT genotype group are summarized in Table 3. Significant variables included age, HbA1c, smoking status and hypertension by univariate Cox regression analysis. While HbA1c (HR, 2.80; 95%CI, 1.07-7.30; $P=0.036$), hypertension (HR, 7.46; 95%CI, 1.38-40.29; $P=0.02$), and hyperlipemia (HR, 12.33; 95%CI, 1.05-145.3; $P=0.046$) were significant in the multivariate model after adjusting for age, smoking status, and drinking status.

Discussion

In our prospective cohort study of subjects with T2DM, the main findings are that: risk indicators of DN were age, smoking, and hyperlipemia were predictors of DN in CC/CT genotype patients, whereas HbA1c, hypertension and hyperlipemia in TT genotype patients.

TGF- β 1 is a pleiotropic cytokine and has been considered as an essential risk factor in the development of DN. Elevated concentration of TGF- β 1 could induce renal hypertrophy and promote excessive accumulation of extracellular matrix (15-17). Exactly these pathological changes contribute to the initiation and progression of DN. During the past few decades TGF- β 1 gene polymorphism was found associated with hepatic carcinoma (18), lung cancer (19), colorectal cancer (20), and various fibrosis disease (21, 22) in many studies. TGF- β T869C gene polymorphism was associated with DN in Chinese (5). Similar results were also found in Egyptian (23). These results were in line with our findings in this prospective cohort study.

The exact mechanism underlying the effect of TGF- β 1 T869C polymorphism on DN susceptibility is not clear. To date, we know that the TGF- β 1 gene is located on chromosome 19 (q13.1-13.3), including 7 exons and 6 introns (24). There are several common known single nucleotide polymorphisms (SNPs) in this gene. Among them, T869C (rs1800470; Leu10/Pro10; T29C, codon10) in exon1 of the TGF- β 1 gene is located at position 10 in the signal peptide. It was supposed that due to substitute C allele for T in T869C, Leucine was replaced by Proline, which accelerated the hydrolysis of TGF- β 1 precursor to a mature TGF- β 1

protein. Consequently, the concentration of TGF- β 1 protein was elevated. In accord with this, several studies have been reported that the number of allele C was positively correlated with serum TGF- β 1 concentration (25, 26). However, further studies will be necessary.

Smoking, hyperglycemia, hypertension, and hyperlipemias are conventional risk factors of developing diabetic nephropathy (27-29). However, we found that some people with serious comorbid conditions would not develop DN. Therefore, we presumed that patients with different genetic background may have different risk factors of developing DN. To confirm this presumption, we evaluated the prognostic risks of DN by Cox regression analysis. In this study, we found that age, smoking, and hyperlipemia was predictors of DN in CC/CT genotype patients, whereas HbA1c, hypertension and hyperlipemia were risk indicators of DN in TT genotype patients. These results were in keeping with our presumption.

Elevated serum levels of low-density lipoprotein cholesterol and triglycerides and low levels of high-density lipoprotein cholesterol are strongly associated with increased risk for macrovascular events (e.g., diabetic nephropathy, myocardial infarction, ischemic stroke, and coronary mortality) among patients with T2DM (29-31). These results are in keeping with our study, that hyperlipemia was a risk predictor of DN in CC/CT genotype group or TT genotype group as well as in total subjects.

In this study, the HbA1c levels and hypertension, two traditional risk indicators of DN were not the risk indicators of DN in CC/CT genotype patients. HbA1c levels are associated with concentrations of plasma glucose, which could stimulate TGF- β 1 expression with a high concentration level (17, 32), and TGF- β 1 protein increase the risk of developing DN. According to preceding hypothesis, T869C, CC/CT genotype is related to high TGF- β 1 protein level without stimulated by high concentration of glucose. In other words, T869C CC/CT genotype is equivalent to the stimulation by glucose. Maybe it is the reason why HbA1c is not the risk indicator of DN for T869C CC/CT genotype patients. Hypertension is a common risk

factor of DN (28, 33), whereas it is not risk indicator of DN in CC/CT genotype. It may be explained by the following reasons. First, the TGF- β 1 T869C genotype polymorphism exerts more marked effects on development of DN than hypertension, and the two factors were correlated in CC/CT genotype subjects (Spearman correlation coefficient = 0.42, $P < 0.001$). When all factors are taken into consideration, the value of the hypertension to predict DN was not superior to that of T869C genotype polymorphism. Secondly, T869C CC/CT genotype weakened the effects of hypertension on developing DN through by TGF- β 1 signal pathway and/or an unknown way by now. In T869C CC/CT genotype group, the samples were relatively small, which might not have sufficient power to detect a small effect size.

In addition, other independent risk factors in our study for the presence of DN in CC/CT genotype patients were older age, smoking. Similar observations were previously made (27, 34-36).

Conclusion

The conclusion was based on relatively small study samples. Larger well-designed studies, involving more relevant variables, should be warranted in future to elucidate the mechanism of the TGF- β 1 T869C genotype polymorphism in DN susceptibility. Despite its limitations, our study may have practical implications. We found that age, smoking, and hyperlipemia were risk indicators for DN in T2DM patients with TGF- β 1 T869C CC/CT genotype, which suggested that these patients should pay more attention to avoid high-fat diet and smoking less. Furthermore, these findings might prompt initiation of appropriate treatment for T2DM patients with CC/CT genotype or TT genotype, respectively.

Ethical Considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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