Cellular and Molecular Biology

Kurnaz-Gömleksiz *et al. Cell. Mol. Biol.*2016, 62 (8): 35-39
ISSN: 1165-158X
doi: 10.14715/cmb/ 2016.62.8.6

Original Research

Can rs3767140 SNP of the perlecan (HSPG2) gene affect the diabetes mellitus through the dyslipidemia?

Ö. Kurnaz-Gömleksiz[1, 2*], B. Tokat[2], E. I. Aslan[2], F. Yanar[2], Z. Ermiş-Karaali[3], O. Öztürk[2], H. Yilmaz-Aydoğan[2]

[1] Department of Medical Biology, Faculty of Medicine, Halic University, Istanbul, Turkey
[2] Department of Molecular Medicine, Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey
[3] Department of Internal Medicine, Haseki Training and Research Hospital, Istanbul, Turkey

Abstract: Perlecan (HSPG2) play an important role in the lipoprotein metabolisms. The G allele of the HSPG2-rs3767140 may affect the binding of heparan sulfate (HS) chains and hence cause loss of HS from the basement membrane. HSPG2-rs3767140 was studied in 60 T2DM patients and 109 healthy controls. In diabetic patients HSPG2-rs3767140 T variant allele carriers (TT+GT) have decreased fasting plasma glucose (FPG) and serum LDL-C levels (p=0.071 and p=0.060, respectively) versus GG genotype carriers. Moreover, in both of the two groups in which the T allele carriers HDL-cholesterol levels tend to be high. We investigated that the HSPG2-rs3767140 promoted to the dyslipidemic phenotype in the type 2 diabetes mellitus (T2DM) patients. We suggest that the HSPG2-rs3767140 might be associated with the decreased FPG and LDL-C and with the increased HDL-C in diabetics. Therefore, the HSPG2-rs3767140 might be a protective for the diabetes mellitus due to its ameliorating effect on the dyslipidemic phenotype.

Key words: Perlecan, HSPG2 gene, Dyslipidemia, Diabetes Mellitus.

Introduction

Dyslipidemia, an essential constituent of the insulin resistance syndrome and type 2 diabetes mellitus (T2DM) (1). One or more lipid disorders in 70- 97 % of diabetic patients has been reported (2, 3). Diabetic dyslipidemia is characterized by increased very low density lipoproteins (VLDLs) and VLDL remnants concentrations, raised serum triglycerides and small dense LDL cholesterol particles (4, 5). In diabetic patients there are different mechanisms for the dyslipidemia. Hyperglycemia and defective insulin action could cause dyslipidemia (5). Some important mechanisms are suggested to have roles in diabetic dyslipidemia such as regulation of lipoprotein lipase (LPL), apoprotein production in the liver by the insulin regulated, the role of the cholesteryl ester transfer protein (CETP) and peripheral role of insulin on adipose tissue and muscles (5).

By reason of the increased triglyceride levels, VLDLs and chylomicron particles stay in bloodstream for longer times. Therefore, transfer of cholesterol esters increase. CETP expedites the carrying of the cholesteryl esters and triglycerides between lipoproteins. It receives triglycerides of VLDL or LDL and switches them for cholesteryl esters present in HDLs, and the other way around. Elevated activity of CETP induces the production of small, dense atherogenic triglyceride-rich LDL particles. These LDL particles are substrates for the hepatic lipase (HL) enzyme. HL has been shown to have increased activity in patients with T2DM (5).

Heparan sulfate proteoglycans (HSPGs) have long and unbranched, sulfated polysaccharide chains (heparan sulfates (HSs)). HSPGs are important components of cell surfaces and extracellular matrix and involved in angiogenesis, coagulation and lipid metabolism (6).

The lipoprotein metabolism is controlled by heparan sulfate proteoglycans (HSPGs) by means of interplays with lipoprotein lipase (LPL) and apolipoprotein B (apoB). LPL catalyzes the hydrolysis of TG and is synthesized in myocytes and adipocytes then transferred and attached to the luminal surface of endothelial cells by way of HSPGs. After binding of LPL to HSPGs, LPL intercedes the degradation and retention of lipoproteins within the subendothelial matrix. Apo B binds to LPL and heparan sulfate (HS) by different binding domains. ApoB is included in the LPL localization in the endothelial surface. Changes in expression of apoB could influence the interactions of LPL with HSPGs or apoB containing lipoproteins. The existence of LPL on endothelial surface and the destruction of TG-rich lipoproteins in bloodstream may be elevated by the increased apo B-HSPG2 (perlecan) binding (6).

Heparan sulfate (HS) was synthesized by the cultured endothelial cells. Glycosaminoglycan chains of HS is an important component of the lipoprotein lipase binding site within endothelial surface. HS fractions extracted from the peripheral blood stimulate the lipoprotein lipase activity. But, it is unknown that the enzyme that is bound to endothelial cells is activated form. Proteoglycans facilitate the lipid metabolism by bringing the enzyme to the cell surface receptor for lipoproteins (7).

Insulin deficiency induces the alteration in hepatocyte proteoglycans, the binding ability to triglyceride rich lipoprotein (TRL) of the HSPG changes. This situation results in higher serum triglyceride levels (8).

Perlecan (HSPG2) gene is located at chromosome 1p36.1 (6). There is a polymorphic BamHI site which

Received March 12, 2016; **Accepted** July 21, 2016; **Published** July 31, 2016

*** Corresponding author:** Özlem Kurnaz-Gömleksiz, Halic University, Faculty of Medicine, Department of Medical Biology, Merkez Mah. Cendere Cad. No:11 Kağıthane, Istanbul, Turkey. Email: ozkurnaz@gmail.com

is mapped to domain I, intron 6. For the HS side chains, a putative attachment site is located in domain I (9). The G allele of the HSPG2 BamHI polymorphism may weaken binding of HS chains and therefore cause loss of HS from the basement membrane (10).

Increasing the binding of LDL to endothelial matrix in vitro through the elimination of HS indicate that HS may intervene with lipoprotein retention and monocyte binding. HS reduces endothelial permeability for LDL (11). In recent studies, it has been reported that HS was decreased in diabetes, the mechanism and importance of this decrement in HS remain unclear. Therefore, based on the potential impact of the HSPG2 BamHI SNP (rs3767140) on lipoprotein lipase enzyme function, the present study is investigated that is this functional genetic variation contributed to the dyslipidemic phenotype in the type 2 diabetic patients.

Materials and Methods

Study participants

We studied 169 individuals in this study: 60 T2DM patients (35 female (58.33%) and 25 male (41.66%), mean age: 58.63±12.29) and 109 non-diabetic controls. Patients were selected from Department of Internal Medicine, Haseki Training and Research Hospital. Diabetes were identified by the WHO definitions and criteria (12).

The patients received a standard inquiry including questions concerning the age, family history, the therapy methods and other medical issues. Patients with a T2DM diagnosis and a history of at least two years of therapy without insulin use were inducted. The measurement of height, weight, and blood pressure were determined in the patients.

Blood pressures were checked as recommended by the American Association (13). The subject lay spine for 10 min where after the blood pressures was measured with a mercury sphygmomanometer. The measurements were performed from the left and the right arm and written down to the nearest 2 mmHg and the mean was determined. The body mass index was calculated using the formula BMI: weight/height^2 (kg/m^2).

The subjects of the control group (38.53 % female, 61.47 % male, mean age: 56.47±10.70) had normal fasting plasma glucose levels and no family history of T2DM.

PCR-Based detection of the HSPG2 rs3767140 polymorphism

Blood samples were collected in tubes with EDTA. Genomic DNA was isolated from the blood by the standard methods. The region of interest for the HSPG2 rs3767140 polymorphism amplified via the primers (forward: 5'-CATGTCCCATGCCCCACGTGT-GCT-3'; and reverse: 5'-ATTGTAGCTGTGGCAG-GCAAACTC-3'). HSPG2 rs3767140 polymorphism was examined by digesting the PCR product with the BamHI enzyme. BamHI digestion resulted in fragments of 150 and 100 bp for the T allele. For the G allele, PCR product (250 bp) was not digested with BamHI. After digestion with BamHI, three genotypes were identified according to the banding types as GG (250 bp), GT (250, 150, and 100 bp) and TT (150 and 100 bp).

Lipid measurement

Blood specimens were collected into plain tubes after an overnight fasting. The centrifugation for 10 min at 1500xg were done to isolate the serum at room temperature and chilled at -20°C. Serum total-C, HDL-C and triglyceride concentrations were determined by enzymatically. LDL-C levels were determined using the Friedewald formula.

Statistical methods

Statistical analyses were examined by the SPSS software package (version 21.0 SPSS Inc., Chicago, IL, U.S.A.). Clinical parameters are given as mean ± SD. Mean values were compared among the groups by unpaired Student's t-test. Differences in the distribution of HSPG2 rs3767140 polymorphism genotypes and alleles of the patients and controls were determined via the Chi-square statistic. Gene counting methods were used for calculating the frequencies of Alleles. Values of $p<0.05$ were accepted statistically significant.

Results

The demographic and biochemical parameters of the study groups were shown in Table-1. As expected,

Table 1 . Demographic characteristics of the Study Groups.

	GROUPS		
	Control (n=109)	**T2DM (n=60)**	**p value**
Age (years)	56.47±10.70	58.63±12.29	0.236
Gender (F/M) (n)	42/67	35/25	0.013
BMI (kg/m^2)	25.85±3.73	26.73±3.73	0.292
Total-C (mmol/L)	5.04±1.17	5.31±1.18	0.330
TG (mmol/L)	2.09±1.43	1.89±0.86	0.428
HDL-C (mmol/L)	1.08±0.27	1.02±0.23	0.336
LDL-C (mmol/L)	3.01±1.07	3.38±0.95	0.119
VLDL-C (mmol/L)	0.71±0.31	0.85±0.35	0.079
Fasting plasma glucose (mg/dL)	112.40±16.02	239.88±136.26	0.001
SBP	119.10±10.05	141.50±28.22	0.000
DBP	71.51±8.34	85.00±17.50	0.000
Smoking (%)	30.3	27.9	0.819

Age, serum lipid and BMI values are shown as Mean+ Std Dev. Statistical analyses were performed by using Students t test for comparison of two groups. BMI: Body Mass Index, Total-C: total-cholesterol, TG: Triglyceride, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, VLDL-C: VLDL-cholesterol, SBP: systolic blood pressure; DBP: diastolic blood Pressure, n: number of subjects.

Table 2. Distributions of HspG2 rs3767140 G>T SNP in the groups.

HspG2	GROUPS	
	Control (n=109)	T2DM (n=60)
Genotypes	n (%)	n (%)
GG	76 (%69.7)	41 (%68.3)
TT	2 (%1.8)	3 (%5.0)
GT	31 (%28.4)	16 (%26.7)
HWE	p=0.565	p=0.396
Alleles		
G	183 (%83.94)	98 (%81.67)
T	35 (%16.06)	22 (%18.33)

n: number of subjects, HWE: Hardy-Weinberg Equilibrium.

the female patients had a higher prevalence (p=0.013). The patients had an increased level of systolic and diastolic blood pressures and HDL-C versus the controls (p=0.001).

In Table 2, the distributions of HSPG2 rs3767140 G>T SNP of genotypes and alleles and Hardy-Weinberg Equilibrium (HWE) were given in the control and diabetic patients groups. The genotype distributions were found suitable for HWE in both of the groups (p>0.05) (Table 2). The frequencies of rs3767140 GG, TT and GT genotypes among the diabetic patients were 0.683, 0.050 and 0.267, respectively; and among the non-diabetic control group they were 0.697, 0.018 and 0.284, respectively. There is no differences for the distributions of HSPG2 rs3767140 SNP between the groups (p>0.05).

In Table 3, the associations between serum lipid concentrations, BMI values, fasting plasma glucose and this polymorphism were examined. In diabetic patients HSPG2 rs3767140 T variant allele carriers (TT+GT) had decreased fasting plasma glucose and serum LDL-K levels (p=0.071 and p=0.060, respectively) compared to wild type homozygote genotype carriers (GG). In other words diabetic patients carrying the GG genotype tend to have higher LDL and fasting glucose levels. Moreover, both the control and the patient group in which the T allele carriers HDL- cholesterol levels tend to be mildly increased, but the results did not reach statistical significance (Table 3). While serum HDL cholesterol levels were 1.12 fold higher in type 2 diabetic subjects with the rare HSPG2 T allele than those with the common HSPG2 GG genotype (1.11±0.25 vs. 0.99±0.22), serum LDL cholesterol levels were 1.22 fold lower in type 2 diabetic subjects with the HSPG2 T allele than those with the HSPG2 GG genotype (2.89±0.91vs. 3.54±0.93) (Figure 1). Also, fasting plasma glucose levels were 1.53 fold lower in the T allele carriers of the type 2 diabetes group than GG genotype (175.20±109.40 vs. 268.00±139.21) (Figure 1). In patients group, we observed no association between blood pressures and this polymorphism. Therefore, our findings indicated that the common HSPG2 GG genotype might increase dyslipidemia risk by causing an increase in serum LDL-cholesterol and a decrement in HDL-cholesterol thus a dyslipidemic phenotype in the type 2 diabetes patient group.

Discussion

Perlecan has been thought to be involved in a lot of complications of diabetes. Decreased perlecan levels have been found in patients with diabetes and in animal models (14). Decreased perlecan levels and lipoprotein abnormalities which were determined in diabetics could be explained by the role of perlecan in the metabolism of lipoproteins (14).

Perlecan regulates the uptake of lipoproteins in the liver. Decreased perlecan levels in liver due to diabetes result in impaired clearance of lipoproteins inducing accumulation of atherogenic particles and increasing the risk of atherosclerosis. Decreased perlecan levels

Table 3. The effects of HspG2 rs3767140 G>T SNP on metabolic parameters in the study groups.

Groups	HspG2 rs3767140 G>T Genotypes		
	GG	TT+GT	p value
Control			
TC (mmol/L)	5.18±1.21	4.47±0.86	0.151
TG (mmol/L)	2.26±1.54	1.48±0.65	0.204
HDL-C (mmol/L)	1.06±0.29	1.14±0.19	0.484
LDL-C (mmol/L)	3.05±1.11	2.83±0.95	0.625
VLDL-C (mmol/L)	0.75±0.32	0.57±0.20	0.191
BMI (kg/m^2)	26.19±3.76	25.03±3.60	0.243
SBP (mmHg)	120.1±11.08	116.5.16	0.268
DBP (mmHg)	72.97±8.37	67.00±6.75	0.048
Fasting Plasma Glucose (mg/dL)	111.50±18.36	116.00±11.42	0.841
T2DM			
TC (mmol/L)	5.39±1.23	5.07±1.06	0.448
TG (mmol/L)	1.84±0.90	2.02±0.70	0.581
HDL-C (mmol/L)	0.99±0.22	1.11±0.25	0.132
LDL-C (mmol/L)	3.54±0.93	2.89±0.91	0.060
VLDL-C (mmol/L)	0.82±0.36	0.92±0.32	0.431
BMİ (kg/m^2)	26.67±4.05	26.88±3.20	0.897
SBP (mmHg)	135.74±25.22	153.46±31.32	0.062
DBP (mmHg)	82.41±15.53	90.38±20.66	0.180
Fasting Plasma Glucose (mg/dL)	268.00±139.21	175.20±109.40	0.071

The results are shown as Mean ± Std Dev. Total-C: total-cholesterol, TG: Triglyceride, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, VLDL-C: VLDL-cholesterol, BMI: Body Mass Index, SBP: systolic blood pressure; DBP: diastolic blood pressure.

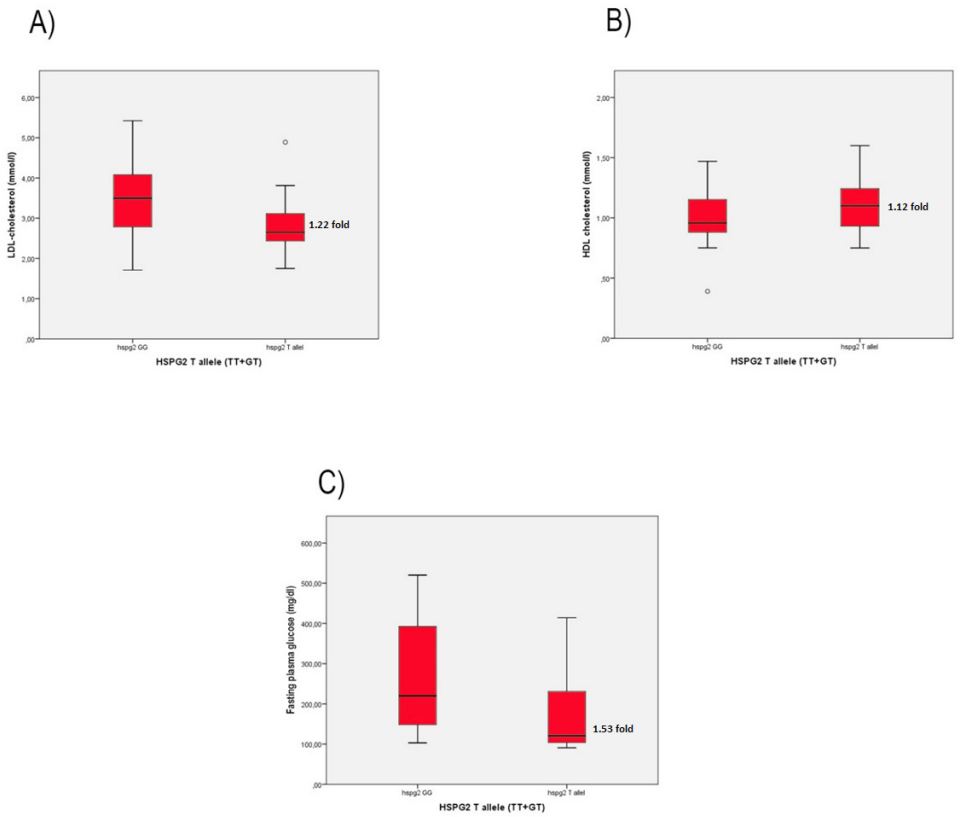

Figure 1. The effects of the HSPG2 T allele on serum HDL cholesterol (a), LDL cholesterol (b) and fasting plasma glucose (c) levels in T2DM patients.

have also been associated with increased formation of atherosclerotic plaque. As indicated by these findings perlecan may play an important role in atherosclerogenesis which is the most significant cause of death in diabetic patients (14).

The patients with diabetes mellitus have a 2-4 fold increased cardiovascular risk versus non diabetics. Additionally, an independent risk factor for the cardiovascular disease is hyperglycemia. One of the potential mechanisms is that increased susceptibility of arterial wall to atherosclerosis by hyperglycemia leads to changes in arterial extracellular matrix components. Proteoglycans (PG) are an important extracellular matrix components. Perlecan, a heparan sulfate proteoglycan (HSPG), is a primary PG that of basement membrane. Heparan sulfate-glycosaminoglycan (HS-GAG) within artery wall, is increased in diabetic patients (15). Most of the arterial HSPGs are produced by endothelial cells and localize in subendothelial matrix (15). LPL binds HSPGs and intercedes the degradation and retention of lipoproteins within the subendothelial matrix. The ancestral G allele of the HSPG2 rs3767140 polymorphism may weaken the binding of HS chains and therefore cause the loss of HS from the basement membrane (6). The loss of HS may affect the binding of LPL. Hence, lipid levels can be affected due to decreased binding activity of LPL. The possible effect of HSPG2 SNP on LPL lead us to investigate the association of HSPG2 SNP with lipid levels in diabetic patients and this study is the first one to investigate this association to the best of our knowledge.

Tao et al. explored the relation of the HSPG2 polymorphism and diabetic nephropathy in Chinese and showed that there were no associations between diabetic patients and non-diabetic control in allele frequencies but obvious difference in genotype frequencies (16).

Cai et al. observed that the plasma total-C, triglyceride, total-C/HDL-C and LDL-C levels were lower in the HSPG2 TT homozygotes versus the GT and GG genotypes even though there was not any significance in the differences of the genotypes in Australian population (6).

In the present study, HSPG2 T allele carriers seemed to have decreased fasting plasma glucose and LDL-C levels both in diabetic and control groups, but this findings were not supported statistically. The finding that is the lower LDL-C levels in T allele carriers is similar to the studies of Cai et al. (6) and Tao et al. (16).

Fujita et al. studied the HSPG2 polymorphism in Japanese patients with diabetes and showed that the frequencies of genotypes and alleles were not differing between the patients with nephropathy and the patients without nephropathy. They suggested that HSPG2 polymorphism was not associated with diabetic nephropathy in Japanese diabetic patients (17).

In a meta-analysis study of Europeans, HSPG2 rs3767140 was found to be related with diabetic nephropathy (OR 0.72 (95% CI 0.59-0.87)), and furthermore with diabetic nephropathy in type 1 diabetes mellitus (OR 0.64 (95% CI 0.49-0.84)) (18).

In Caucasian IDDM patients from Denmark and UK, the G allele of the HSPG2 BamHI polymorphism have a risk for diabetic nephropathy (9).

Hence, our findings may support that in the type 2 diabetes mellitus patients T allele has a decreasing effect on serum LDL-C and fasting plasma glucose levels and may protect the development of dyslipidemia and diabetes mellitus.

As a conclusion, this study is the first one conducted on Type 2 Diabetes Mellitus in the Turkish population

about the association between the HSPG2 gene polymorphism and the serum lipid levels. Our findings suggest that HSPG2 rs3767140 might be associated with decreased fasting plasma glucose and LDL-C, and with increased HDL-C in diabetic patients. Therefore HSPG2-rs3767140 might play a protective role in diabetes mellitus as a result of its ameliorating effect on the dyslipidemic phenotype due to the polymorphic perlecan molecule.

Acknowledgements
I would like to thank Alison P. Eronat for the English corrections.

References

1. Betteridge DJ. Lipid control in patients with diabetes mellitus. Nat Rev Cardiol 2011; 8:278-290.
2. Haffner SM. Dyslipidemia management in adults with diabetes. Diabetes Care 2004; 27:68-71.
3. Ergin E, Akın S, Kazan S, Erdem ME, Tekçe M, Aliustaoğlu M. Lipid Profile of Diabetic Patients: Awareness and the Rate of Treatment Success. Kartal TR 2013; 24(3):157-163.
4. Muačević-Katanec D, Reiner Ž. Diabetic Dyslipidemia or 'Diabetes Lipidus? Expert Rev Cardiovasc Ther 2011; 9(3):341-348.
5. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nat Rev Endocrinol 2009; 5:150-159.
6. Cai H, Wang XL, Wilcken DEL. Genetic polymorphism of heparan sulfate proteoglycan (Perlecan, HSPG2), lipid profiles and coronary artery disease in the Australian population. Atherosclerosis 2000; 148(1):125–129
7. Gallagher JT, Lyon M and Steward WP. Structure and function of heparan sulphate proteoglycans. Biochem J 1986; 236:313-325
8. Bishop JR, Foley E, Lawrence R, Esko JD. Insulin-dependent Diabetes Mellitus in Mice Does Not Alter Liver Heparan Sulfate. J Biol Chem 2010; 285(19):14658-14662.
9. Hansen PM, Chowdhury T, Deckert T, Hellgren A, Bain SC, Pociot F. Genetic variation of the heparan sulfate proteoglycan gene (perlecan gene): association with urinary albumin excretion in IDDM patients. Diabetes 1997; 46(10):1658-1659.
10. Chowdhury TA, Dyer PH, Kumar S, Barnett AH, Bain SC. Genetic Determinants of Diabetic nephropathy. Clinical Science 1999; 96:221-230
11. Tran-Lundmark K, Tran PK, Paulsson-Berne G, Fridén V, Soininen R, Tryggvason K, et al. Heparan sulfate in perlecan promotes mouse atherosclerosis: roles in lipid permeability, lipid retention, and smooth muscle cell proliferation. Circ Res 2008; 103(1):43–52.
12. WHO. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva, World Health Org. 2006, pp. 1–46.
13. Kirkendall WM, Feianleib M, Freis ED, Mark AL. Recommendation for human blood pressure determination of by sphygmomanometers. Circulation 1980; 62:1146.
14. Conde-Knape K Heparan sulfate proteoglycans in experimental models of diabetes: a role for perlecan in diabetes complications. Diabetes Metab Res Rev 2001; 17:412–421.
15. Vogl-Willis CA and Edwards IJ. High glucose-induced alterations in subendothelial matrix perlecan leads to increased monocyte binding. Arterioscler Thromb Vasc Biol 2004; 24:858-863.
16. TAO Y, JIAWEI C, JIE S. A study for HSPG gene polymorphism in Chinese type 2 diabetic nephropathy. Chinese J Diabetes 2001; 2:005.
17. Fujita H, Narita T, Meguro H, Ishii T, Hanyu O, Suzuki K et al. Lack of association between the heparan sulfate proteoglycan gene polymorphism and diabetic nephropathy in Japanese NIDDM with proliferative diabetic retinopathy. Renal Failure 1999; 21(6):659-664.
18. Mooyaart AL, Valk EJJ, van Es LA, Bruijn JA, de Heer E, Freedman BI et al. Genetic associations in diabetic nephropathy: A Meta-analysis. Diabetologia 2011; 54: 544-553.