Interaction of MTHFR C677T polymorphism with smoking in susceptibility to diabetic nephropathy in Chinese men with type 2 diabetes

Liang Ma1 · Yongwei Jiang1 · Xiaomu Kong2 · Qian Liu1 · Hailing Zhao3 · Tingting Zhao3 · Yongtong Cao1 · Ping Li3

Received: 3 July 2018 / Revised: 2 September 2018 / Accepted: 17 October 2018 / Published online: 5 November 2018
© The Author(s) under exclusive licence to The Japan Society of Human Genetics 2018

Abstract
We investigated the interaction of MTHFR C677T polymorphism (rs1801133) with smoking in susceptibility to diabetic nephropathy (DN) in Chinese men with type 2 diabetes mellitus (T2DM). We studied 655 Chinese men with T2DM, who were divided into two groups (321 with DN and 334 without DN). The genotype of MTHFR C677T polymorphism was detected by real-time polymerase chain reaction. MTHFR TT genotype carried a higher risk of DN compared with the CC genotype (OR = 2.05; P = 0.002). The T allele showed marked association with DN development in patients who smoked, using additive, recessive, and dominant models (OR = 1.60, 1.83, and 1.88, respectively; P = 0.006, 0.002, and 0.04, respectively), which was not observed in the nonsmoking group. Patients with TT and CT genotypes, who smoked had a higher risk of DN compared with the control group (non-smoking with CC genotype; OR = 3.73 and 2.28, respectively; P < 0.001 and P = 0.004, respectively), whereas the other groups were not observed. In conclusion, the T allele of rs1801133 may be a risk factor for DN in Chinese men with T2DM, and synergy appears to exist between the MTHFR rs1801133 and smoking in susceptibility to DN.

Introduction
Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes mellitus (DM) [1]. Type 2 DM (T2DM) is becoming increasingly prevalent worldwide. Among the Chinese adult population, the estimated overall prevalence of diabetes was 10.9% in 2013 [2]. Approximately 30–40% of T2DM patients develop DN. The determinants of DN are complex, and it includes genetic and environmental factors. There appears to be an inherited predisposition for DN, and many investigations on the familial clustering of DN in T2DM and the heritability of DN provide compelling evidence that genetic factors contribute to DN susceptibility [3, 4]. Furthermore, a few candidate genes have been reproducibly associated with DN. Genetic studies may provide valuable information regarding the pathobiology of DN and potential targets for its treatment.

It is reported that the level of plasma homocysteine (Hcy) might be closely linked with progression of DN [5]. Hcy metabolism is regulated by methylenetetrahydrofolate reductase (MTHFR). MTHFR C677T polymorphism (rs1801133) changes amino acid 222 from alanine to valine (A222V), which causes reduced MTHFR activity and elevated Hcy level, and MTHFR C677T polymorphism has been studied as a candidate genetic risk factor for DN. Compared with MTHFR CT or CC genotype, individuals with TT genotype have higher plasma Hcy levels [6]. Many studies about the association between MTHFR C677T polymorphism and DN have shown heterogeneous results [7–9], which might have been influenced by different methods and insufficient statistical power.

In recent years, greater emphasis has been laid on the joint effects of genetic and environmental factors on
complex disease traits. Prospective and retrospective studies have found that smoking is closely related to DN progression [10–13]. The influence of smoking on association between the MTHFR C677T polymorphism and the risk of DN has received little attention.

Based on these observations, we explored the association of smoking and MTHFR C677T polymorphism with DN in a cohort of men with T2DM in Beijing, China, to evaluate the combined effect of MTHFR C677T and smoking on DN progression.

Materials and methods

Patients

This was a case–control study of 655 male patients with T2DM, diagnosed according to the World Health Organization 1999 criteria [14]. They were all hospitalized at the China–Japan Friendship Hospital, Beijing, China between August 2016 and December 2017. This study was approved by the hospital Institutional Ethics Committee. Written informed consent was obtained from all patients.

DN patients

Individuals who had a history of DN were defined as the DN group (n = 321). Inclusion criteria for the DN group were: diagnosis of T2DM; 24-h proteinuria > 500 mg/24 h, or albumin creatinine ratio (ACR) > 30 mg/g. Exclusion criteria were: known proteinuria before onset of diabetes; other primary or secondary renal diseases (e.g., IgA nephropathy, membranous nephropathy, lupus nephritis, obstructive renal disease, renal stone disease, and acute urinary tract infection); or malignancy.

T2DM patients

Individuals who were diagnosed with T2DM for ≥7 years and without DN were defined as controls (n = 334). Controls were unlikely to have DN in the future, which increased the sensitivity in detecting the association between MTHFR C677T and DN, and had ACR < 30 mg/g. We excluded patients with other primary or secondary renal diseases (e.g., IgA nephropathy, membranous nephropathy, lupus nephritis, obstructive renal disease, renal stone disease, and acute urinary tract infection), or malignancy.

Smokers consisted of individuals who smoked at least one cigarette/day for a minimum of 2 years. As most of the women were non-smokers, only men were included in the present study. The method of data collection was the same as in our previous study [15].

Genomic DNA extraction and genotyping

Genomic DNA was extracted from EDTA-treated whole blood using QIAamp DNA Blood Mini Kit (Qiagen). The quality and concentration of DNA was determined using the NanoDrop 1000 spectrophotometer (ThermoScientific). Genotyping of MTHFR C677T was performed by polymerase chain reaction (PCR) using TaqMan SNP Genotyping Assay (Applied Biosystems). The MTHFR primer sequences were forward: 5′-GGCTGACCTGAAAGCA CTTGAA-3′, reverse: 5′-AGAAAGCTGCCTGAT GATGAA-3′. The probe sequences were FAM-5′- TCTGGGAGTGC-3′-MGB; VIC-5′-CTGCGGAGCC GA-3′-MGB. The primers and probes were designed by Applied Biosystems. The amplification conditions consisted of denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s, and then by annealing and elongation at 65 °C for 60 s. To verify the genotypes, PCR products from 50 patients were randomly selected for DNA sequencing. The forward primer used for PCR was 5′- GTCTCTTCATCCCTCGCCTT-3′ and the reverse primer was 5′-GAACCTCAGAATCTCAGCAC-3′. The products were analyzed by ABI 3500 (Applied Biosystems).

Statistical analysis

The baseline characteristics age, body mass index (BMI), blood pressure, duration of diabetes, hemoglobin A1C, total cholesterol (TC), high-density lipoprotein–cholesterol (HDL-C), low-density lipoprotein–cholesterol (LDL-C), triglyceride (TG), and Hcy showed a non-Gaussian distribution, and the data were presented as median (interquartile range). The differences in clinical characteristics between the DN and control groups were analyzed by Wilcoxon signed rank test. Genotype distribution and allelic frequency were analyzed using the $\chi^2$ test. A goodness of fit $\chi^2$ test was used to evaluate whether the genotypic distribution of the MTHFR C677T polymorphism was in accordance with Hardy–Weinberg equilibrium in the DN and control groups.

We also used multivariate logistic regression to analyze the association between MTHFR C677T polymorphism and DN adjusted for age, BMI, duration of diabetes, history of hypertension, smoking, and TC and TG levels using three genetic models (additive, recessive, and dominant models). TT and CT were coded as 1 while CC was coded as 0 in the dominant model; TT was coded as 1 while CC and CT were coded as 0 in the recessive model; and TT, CT, and CC were coded as 2, 1, and 0, respectively, in the additive model. Data were analyzed with SPSS version 17.0 software. P < 0.05 was considered a significant difference, and P < 0.01 was considered a highly significant difference.
Results

General characteristics of the study population

We included 321 DN patients (DN group) and 334 T2DM patients (control group), and all 655 participants were male. BMI, history of hypertension, smoking status, blood pressure, Hcy, LDL-C, and TG in the DN group were significantly higher than those in the control group ($P<0.05$) (Table 1). However, HDL-C in the DN group was significantly lower compared with that in the control group ($P<0.05$) (Table 1). There were no significant differences in age, duration of diabetes, hemoglobin A1C, TC, and LDL-C between the two groups ($P>0.05$).

Genotypic and allele frequency of MTHFR C677T polymorphism

Genotypic and allelic frequencies for the MTHFR C677T polymorphism are presented in Table 2. The distribution of allele frequencies of the polymorphism among the study population was in accordance with the Hardy–Weinberg equilibrium in both the DN and control groups. The T allele frequency of MTHFR C677T in the DN group (59.2%) was significantly higher than that in the control group (51.05%) ($P<0.01$). Genetic distribution of MTHFR C677T (CC, CT, and TT) in the two groups differed significantly ($P<0.01$) (Table 2).

Association between MTHFR C677T polymorphism and DN

To confirm the association between the risk of DN and MTHFR C677T polymorphism, further multivariate logistic regression analysis was performed. The additive, dominant, and recessive models were significantly different in participants with DN than in the control group ($P=0.003, 0.005,$ and 0.03, respectively) (Table 3). TT genotype significantly increased the risk of DN compared with the CC genotype (OR = 2.05, 95% CI = 1.30–3.24, and $P=0.002$), and CT genotype was also found to increase the risk of DN compared with the CC genotype (OR = 1.62, 95% CI = 1.30–3.24, and $P=0.002$).

Table 1 The characteristics of the study participants

| Variables               | DN (n = 321) | Control (n = 334) | P  |
|-------------------------|--------------|------------------|----|
| Age, y                  | 60.0 (53.0, 69.0) | 61.0 (54.0, 68.0) | 0.77|
| BMI, Kg/m²              | 26.26 (24.39, 28.70) | 25.31 (23.0, 27.64) | <0.01|
| Duration of diabetes, y | 15.0 (9.0, 21.0) | 14.0 (10.0, 19.0) | 0.43|
| History of hypertension (%) | 77.9 (250/321) | 51.5 (172/334) | <0.01|
| Smoking (%)             | 53.0 (170/321) | 39.8 (133/334) | <0.01|
| SBP (mm Hg)             | 138.00 (125.00,150.00) | 126.00 (120.00,140.00) | <0.01|
| DBP (mm Hg)             | 80.0 (75.0, 86.0) | 80.0 (70.0, 81.0) | <0.01|
| A1C (%)                 | 7.7 (6.7, 9.4) | 7.9 (6.8, 9.2) | 0.45|
| Hcy (μmol/L)            | 13.52 (11.16, 16.41) | 11.57 (9.65, 13.42) | <0.01|
| TC (mmol/L)             | 3.99 (3.30, 5.02) | 4.12 (3.50, 4.75) | 0.77|
| HDL-C (mmol/L)          | 0.92 (0.77, 1.13) | 0.97 (0.81, 1.19) | 0.03|
| LDL-C (mmol/L)          | 2.39 (1.82, 3.08) | 2.40 (1.93, 2.95) | 0.75|
| TG (mmol/L)             | 1.62 (1.12, 2.55) | 1.49 (1.02, 2.20) | 0.03|

A1C hemoglobin A1C, BMI body mass index, DBP diastolic blood pressure, Hcy homocysteine, HDL-C high-density lipoprotein–cholesterol, LDL-C low-density lipoprotein–cholesterol, SBP systolic blood pressure, TC total cholesterol, TG triglyceride

*Data are shown as median (interquartile range) or %.

Table 3 Odds ratios for DN under three genetic models

| Genetic models | Unadjusted | Adjusted* | Adjusted** |
|----------------|------------|-----------|------------|
|                | OR (95% CI) | P         | OR (95% CI) | P         | OR (95% CI) | P         |
| Additive       | 1.41 (1.13–1.77) | 0.003      | 1.40 (1.11–1.76) | 0.004      | 1.46 (1.14–1.85) | 0.002  |
| Recessive      | 1.44 (1.03–2.02) | 0.03       | 1.44 (1.02–2.03) | 0.04       | 1.55 (1.07–2.23) | 0.02    |
| Dominant       | 1.76 (1.18–2.62) | 0.005      | 1.73 (1.16–2.59) | 0.007      | 1.76 (1.15–2.68) | 0.009   |
| CT vs. CC      | 1.62 (1.06–2.45) | 0.02       | 1.59 (1.04–2.43) | 0.03       | 1.57 (1.01–2.45) | 0.047   |
| TT vs. CC      | 2.05 (1.30–3.24) | 0.002      | 2.01 (1.27–3.21) | 0.003      | 2.15 (1.32–3.51) | 0.002   |

CI confidence interval, DN diabetic nephropathy, OR odds ratio
*Adjusted for age, BMI
**Adjusted for age, BMI, duration of diabetes, history of hypertension, smoking, TC and TG levels
The data indicated that the T allele of MTHFR C677T polymorphism was a potential risk factor for DN progression. After adjusting for age, BMI, duration of diabetes, history of hypertension, smoking, and TC and TG levels, results were similar.

Association of MTHFR C677T polymorphism with DN risk in smoking and nonsmoking groups

Participants were divided into two groups based on smoking status. The additive, recessive, and dominant genetic models showed significant associations with DN in the smoking group in the unadjusted models ($P = 0.006$, $0.02$ and $0.04$, respectively), indicating that the T allele carriers in the smoking group were at higher risk of DN. However, no significant association was observed in the nonsmoking group ($P = 0.19$, $0.66$, and $P = 0.09$, respectively) in the unadjusted models. After multivariate adjustment, we achieved similar results in the two groups (Table 4).

Association of the combined effect of MTHFR C677T polymorphism and smoking on DN risk

Combination of MTHFR TT or CT genotype and smoking carried a higher risk of DN compared with the control group (non-smoking with CC genotype; OR = 3.73 and 2.28; $P < 0.001$ and $P = 0.004$), whereas the combined effects of MTHFR CC genotype and smoking compared with those of MTHFR CT or TT genotype and non-smoking were not observed. After adjusting for age, BMI, duration of diabetes, history of hypertension, TC and TG levels, results were similar (Table 5).

Discussion

In this study, we systematically explored the effects of the MTHFR C677T polymorphism, smoking, and their interactions on the development of DN among Chinese men with T2DM. This is one of the few studies to describe the association of the combined effect of MTHFR C677T polymorphism and smoking on DN. The effect of the T allele of MTHFR C677T polymorphism resulted in a higher risk of DN in all three genetic models (additive, dominant, and recessive). When participants were divided into two groups based on smoking status, significant association of T allele of MTHFR C677T polymorphism with the risk of DN was observed only in the smoking group in all three genetic models, whereas in the nonsmoking group no association was observed. Furthermore, the combined effect of the MTHFR TT and CT genotype with smoking resulted in a higher risk of DN compared with the control group (non-smoking with CC genotype). We conclude that the T allele of MTHFR C677T appears to impart susceptibility to DN in Chinese men with T2DM in interaction with smoking.
It has been shown that high plasma Hcy is an independent risk factor for DN progression in T2DM patients [16], which is associated with MTHFR C677T polymorphism [17]. This was also shown in our previous study [15]. Elevated Hcy levels, predominantly caused by polymorphisms in key enzyme genes involved in Hcy metabolism, is a risk factor for DN. Furthermore, the frequency of the CT and TT genotypes was 43.9% and 23.2%, respectively, in the Chinese population, which is higher compared with many other populations worldwide [18, 19]. Higher Hcy levels are found in the Chinese population due to the MTHFR C677T polymorphism [20]. In the present study, 48 (15%), 166 (51.7%), and 107 (33.3%) patients in the DN group had the CC, CT, and TT genotypes of MTHFR, respectively, while 79 (23.7%), 169 (50.6%), and 86 (25.7%) in the control group had these genotypes, respectively. The genotype distribution differed significantly in two groups. The T allele frequency in the DN group was significantly higher than in the control group, which indicated that the increased T allele frequency might be related to the DN incidence.

In order to detect the association between MTHFR C677T polymorphism and DN, we performed logistic regression analysis using additive, recessive, and dominant genetic models. Our results indicated that the T allele of MTHFR C677T polymorphism was a potential risk factor in DN progression, and TT genotype was more likely to promote DN development compared with CC genotype in unadjusted models, which was in accordance with another study [9]. Previous meta-analyses have suggested a potential association between the MTHFR C677T polymorphism and DN susceptibility; however, the results of these studies were inconsistent [7–9, 21]. Our data further revealed that the MTHFR C677T polymorphism was closely related to DN development in Chinese men with T2DM.

Recently, growing emphasis has been attached to the combined influence of genetic and environmental factors on DN development [22]. One study showed that the MTHFR C677T polymorphism might be related to DN progression in the Chinese population, and gene–environment interactions should be further studied [23]. Smoking, as a novel risk factor, has been considered to play a key role in DN development [13, 24, 25], and smoking might influence DN progression induced by MTHFR polymorphism. In our study, the number of smokers was higher in the DN group than the controls, and all the participants were male. In order to explore the relationship between smoking and MTHFR polymorphism-induced DN progression, we analyzed smoking status stratification. MTHFR polymorphism was significantly associated with DN in the smoking group using the additive, recessive, and dominant genetic models. However, no significant association was observed in the nonsmoking group. The results indicated that smoking has an important effect on the association between MTHFR C677T polymorphism and DN progression.

Although the precise mechanisms by which smoking and MTHFR C677T polymorphism interact to influence DN development remain unclear, it has been shown that smoking might have a major impact on renal structure and function. First, nephrotoxic effects of smoking have been reported, including production of pro-inflammatory factors, enhanced oxidative stress, endothelial dysfunction, glomerulosclerosis, and tubular atrophy [26, 27]. Second, glycotoxins in cigarette smoke can cause production of advanced glycation end products [28], which can induce vascular nephropathy [29]. Third, smoking is related to insulin resistance in patients with diabetes [30] and accelerates renal atherosclerosis [31]. Furthermore, in order to study the joint effect of MTHFR polymorphism and smoking risk factors on DN progression, we compared the ORs for the combined effect of MTHFR C677T and smoking on DN. Our data showed that the smokers with TT and CT genotypes had a higher risk of DN compared with the control group, whereas no combined effect was observed among the remaining three groups (smoking with CC genotype, or non-smoking with CT or TT genotype). Therefore, the combined interaction of smoking and MTHFR genotype might increase the risk of DN in Chinese men with T2DM.

There were some limitations to this study. First, the study population was small when stratified by smoking status, which might have influenced the statistical power to detect associations. Second, although we identified the rs1801133 polymorphism in the MTHFR gene as a susceptibility variant in Chinese men with T2DM, screening more loci within the MTHFR gene to clarify the interaction between polymorphisms and DN is clearly warranted. Third, the result is warranted to be replicated in another independent cohort in the future. Finally, more environmental risk factors should be investigated in future studies, and the precise biological mechanism of the combined effects of smoking and MTHFR C677T polymorphism on susceptibility to DN needs further elucidation.

In conclusion, this study suggested that the T allele of MTHFR C677T is associated with DN development. Additionally, these results suggest that a synergistic effect of MTHFR C677T and smoking appears to be significantly associated with development of DN in the Chinese men with T2DM. Our findings provide a further insight into the gene–environment relationships involved in DN pathogenesis. Therefore, future large, well-designed studies in Chinese, and other populations are needed to validate our findings. Furthermore, based on our observations, smoking cessation should be considered in carriers with high genetic risk, which can selectively decrease the risk of DN.
Acknowledgements This study was supported by the the National Natural Science Foundation of China (Grant no: 81703892, 81503418).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Remuzzi G, Schiappati A, Ruggenenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. N Engl J Med. 2002;346:1145–51.
2. Limin W, Pei G, Mei Z, Zhengjing H, Dandan Z, Qian D, et al. Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. JAMA. 2017;317:2515–23.
3. Pezzolesi MG, Krolevski AS. The genetic risk of kidney disease in type 2 diabetes. Med Clin North Am. 2013;97:91–107.
4. Skrunes R, Svarstad E, Reisaeter AV, Vikse BE. Familial clustering of ESRD in the Norwegian population. Clin J Am Soc Nephrol. 2014;9:1692–700.
5. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005;28:164–76.
6. Wei W, Yujia W, Fangqi G, Weihua Z, Songling F. MTHFR C677T polymorphism and risk of congenital heart defects: evidence from 29 case-control and TDT studies. PLoS One. 2013;8:e58041.
7. Niu W, Qi Y. An updated meta-analysis of methylenetetrahydrofolate reductase gene 677C/T polymorphism with diabetic nephropathy and diabetic retinopathy. Diabetes Res Clin Pract. 2012;95:110–8.
8. Wei-Wei C, Liu, Z, Ying-Shui Y, Hong, S, Yue-Long J, Yan C. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and susceptibility to diabetic nephropathy in Chinese type 2 diabetic patients: a meta-analysis. Ren Fail. 2013;35:1038–43.
9. Zhou TB, Drummen GP, Jiang ZP, Li HY. Methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism and diabetic nephropathy susceptibility in patients with type 2 diabetes mellitus. Ren Fail. 2015;37:1247–59.
10. Sawicki PT, Didjurgeit U, Mühlhauser I, Bender R, Heinemann L, Berger M. Smoking is associated with progression of diabetic nephropathy. Diabetes Care. 1994;17:126–31.
11. Chuaahirun T, Khanna A, Kimball K, Wesson DE. Cigarette smoking and increased urine albumin excretion are interrelated predictors of nephropathy progression in type 2 diabetes. Am J Kidney Dis. 2003;41:13–21.
12. Maija F, Valma H, Carol F, Lena T, Johan W, Nina T, et al. Smoking and progression of diabetic nephropathy in patients with type 1 diabetes. Acta Diabetol. 2016;53:525–33.
13. Jose MJ, Varkey V, Chandri R, Zubaia PA, Maliekkal J. The role of smoking as a modifiable risk factor in diabetic nephropathy. J Assoc Physicians India. 2016;64:34–38.
14. WHO. Report of a WHO consultation, Part 1: Diagnosis and classification of diabetes mellitus, Geneva, 1999.
15. Liu P, Yongwei J, Xiaolu K, Meihua Y, Tingting Z, Hailing Z, et al. Synergistic effect of the MTHFR C677T and EPHX2 G860A polymorphism on the increased risk of ischemic stroke in Chinese type 2 diabetic patients. J Diabetes Res. 2017;2017:6216205.
16. Huan W, Kai C, Ke X, Shixin X. Association between plasma homocysteine and progression of early nephropathy in type 2 diabetic patients. Int J Clin Exp Med. 2015;8:11174–80.
17. Sibani S, Christensen B, O’Ferrall E, Saadi I, Hoïou-Tim F, Rosenblatt DS, et al. Characterization of six novel mutations in the methylenetetrahydrofolate reductase (MTHFR) gene in patients with homocystinuria. Hum Mutat. 2000;15:280–7.
18. Wilcken B, Bamforth F, Li Z, Zhu H, Ritvanen A, Renlund M, et al. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas worldwide. J Med Genet 2003;40:619–25.
19. Boyi Y, Yuyan L, Yongfang L, Shujun F, Xueyuan Z, Xiangxiang L, et al. Geographical distribution of MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in China: findings from 15357 adults of Han nationality. PLoS One. 2013;8:e57917.
20. Xianhui Q, Jianping L, Yimin C, Zeyuan L, Junbo G, et al. Association between MTHFR C677T polymorphism and MTR A2756G polymorphisms and the homocysteine lowering efficacy of different doses of folic acid in hypertensive Chinese adults. Nutr J. 2012;11:2.
21. Zintzaras E, Uhlig K, Koukoulis GN, Papathanasiou AA, Stafides I. Methylenetetrahydrofolate reductase gene polymorphism as a risk factor for diabetic nephropathy: a meta-analysis. J Hum Genet. 2007;52:881–90.
22. Rutter M, Moffitt TE, Caspi A. Gene-environment interplay and psychopathology: multiple varieties but real effects. J Child Psychol Psychiatry. 2006;47:226–61.
23. Xuan X, Xiao-Kun L,Xiao X, Dan-Ping Q, Dao-Yuan Z,Jian-Guang H, et al. Association between MTHFR C677T polymorphism and diabetic nephropathy in the Chinese population: An updated meta-analysis and review. Nephrol (Carlton). 2016;21:5–12.
24. Arvind CV. Smoking in diabetic nephropathy: sparks in the fuel tank? World J Diabetes. 2012;3:186–95.
25. Yeom H, Lee JH, Kim HC, Suh I. The association between smoking tobacco after a diagnosis of diabetes and the prevalence of diabetic nephropathy in the Korean male population. J Prev Med Public Health. 2016;49:108–17.
26. Caimi G, Hoppes E, Montana M, Carollo C, Calandrino V, Incalcaterra E, et al. Nitric oxide metabolites (nitrate and nitrite) in several clinical condition. Clin Hemorheol Microcirc. 2014;56:359–69.
27. Salvatore SP, Troxell ML, Hecox D, Sterling KR, Seshan SV. Smoking-related glomerulopathy: expanding the morphologic spectrum. Am J Nephrol 2015;41:66–72.
28. Cerami C, Founds H, Nicholl I, Mitsuhashi T, Giordano D, Vanpatten S, et al. Tobacco smoke is a source of toxic reactive glycation products. Proc Natl Acad Sci USA. 1997;94:13915–20.
29. Lei L, Yongsheng H, Wei R, Jielong J, Peng W, Zhao H. Advanced glycation endproducts affect the cytoskeletal structure of rat glomerular endothelial cells via the Ras?related C3 botulinum toxin substrate 1 signaling pathway. Mol Med Rep. 2015;11:4321–6.
30. Toshiaki O, Masanori I, Hiroki F, Shinako K, Hitoshi I, Tamaki J, et al. Dose- and time-dependent association of smoking and its nicotine component on advanced glycation endproducts. Proc Natl Acad Sci USA. 2006;103:1663–7.