Int J Clin Exp Pathol 2016;9(10):10522-10528
www.ijcep.com /ISSN:1936-2625/IJCEP0028711

Original Article

Association of interleukin-18 polymorphisms and the susceptibility to diabetic nephropathy

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Received March 19, 2016; Accepted August 1, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: We firstly investigated the association between *IL-18* -137G/C and -607C/A genetic polymorphisms and the risk of developing diabetic nephropathy in a Chinese Han population. A total of 155 patients diagnosed with type 2 diabetes mellitus and 320 healthy controls were recruited from the First Affiliated Hospital of Xinxiang Medical University between January 2013 and March 2015. Genotyping of *IL-18* -137G/C and -607C/A polymorphism was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). As determined by multiple logistic regression analysis, individuals carrying GC genotype of *IL-18* -137G/C were correlated with an elevated risk of diabetic nephropathy in comparison with the GG genotype, and the crude and adjusted ORs were 2.31 (1.49-3.56) and 2.54 (1.63-4.11), respectively. Moreover, the C allele of *IL-18* -137G/C was associated with risk of developing diabetic nephropathy in comparison with G allele, and the crude and adjusted ORs were 1.81 (1.25-2.59) and 2.16 (1.36-2.85), respectively. In conclusion, our study suggests that *IL-18* -137G/C is associated with development of diabetic nephropathy in the Chinese population.

Keywords: *IL-18*, -137G/C, -607C/A, polymorphism, diabetic nephropathy

Introduction

Diabetic nephropathy is a major cause of end-stage renal disease and high mortality in diabetic patients [1]. Diabetic nephropathy was increasing rapidly worldwide. Although improvements in early detection and treatments have decreased the mortality rate of diabetic nephropathy in recent years, the lack of effective preventative measures for diabetic nephropathy remains a major public health problem. The development of diabetic nephropathy occurs over a long period of time, involves multifactorial processes [2]. Besides, genetic susceptibility may also be an important determinant of both the incidence, progression and severity of diabetic nephropathy [3]. However, the molecular pathogenesis of diabetic nephropathy is not fully understood. Therefore, it is necessary to identify novel molecular targets involved in the pathogenesis of diabetic nephropathy.

Plasma levels of some cytokines, such as ICAM-1, interleukin-18 (*IL-18*) and *IL-1*, in patients with diabetic nephropathy are higher than those in healthy population. IL-18 shows high secretion and immune response under hyperosmotic stress condition [4]. Previous study has reported that the plasma level of *IL-18* in diabetic nephropathy is higher than those in healthy individuals [5, 6]. *IL-18* gene is located at 11q22.2-22.3, including six exons and five introns. Previous studies have indicated that polymorphisms in IL-18 could affect the plasma level of this protein, and thus influence the expression level of TFN-γ and immunologic function of TH1 [7]. Two common promoters in the *IL-18* (-137G/C and -607C/A) could influence the function of this protein, and only one previous study has reported the association of *IL-18* -137G/C and -607C/A polymorphism with the development of risk of cardiovascular diseases in diabetic nephropathy patients [8]. In the present study, we firstly investigated the association between *IL-18* -137G/C and -607C/A genetic polymorphism and the risk of developing diabetic nephropathy in a Chinese Han population.

Table 1. Primer sequences, restriction enzymes, restriction digest products of *IL-18* -137G/C and -607C/A

IL-18	Primer sequences (5'-3')	Amplification products	Restriction enzymes	Restriction digest products
-137G/C	TTGTAACATTGTAGGAATTACC ATGTAATATCACTATTTTCATGAGA	256 bp	BfuCl	CC:256 bp. GC: 256 bp, 229 bp and 27 bp. GG:229 bp and 27 bp.
-607C/A	CCCTCTCCCCAAGCTTACTT TTCAGTGGAACAGGAGTCCA	137 bp	MseI	AA: 91 bp and 46 bp. CA: 137 bp, 91 bp and 46 bp. CC: 137 bp.

Material and methods

Subjects

A hospital-based case-control study was performed. A total of 155 patients diagnosed with type 2 diabetes mellitus were recruited from the First Affiliated Hospital of Xinxiang Medical University between January 2013 and March 2015. Patients with type 2 diabetes mellitus were confirmed according to the criteria from WHO in 1999 [9]. Patients were excluded who had no history of type I diabetes mellitus, malignant tumors, chronic or acute infection diseases, other endocrine diseases except for type 2 diabetes mellitus and end-stage liver diseases.

A total of 320 control subjects were selected from individuals who visited the outpatient clinics or obtained regular health check-up at the same hospital during the same time period. All control subjects were Chinese Han population, and they were confirmed to be free of a history of type 2 diabetes mellitus, nephropathy or endocrine diseases, as well as end-stage liver diseases.

The demographic and lifestyle characteristics of all study subjects were collected from a structured questionnaire, and the information regarding age, sex, diabetic duration, hypertension and body mass index (BMI). Clinical information was collected from medical records, including systolic and diastolic blood pressure and the levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein-C (HDL-c), low-density lipoprotein-C (LDL-c), and creatinine, as well as duration of diabetes. Written informed consents were obtained from all diabetic nephropathy patients and controls before enrollment. The performance of our study was approved by the Institutional Review Board of the First Affiliated Hospital of Xinxiang Medical University. The performance of our study was in agreement with the declaration of Helsinki.

Genotyping analysis

Five ml blood sample was taken from all participants in EDTA-containing tube for total genomic DNA extraction after enrollment. Genomic DNA was extracted using QIAamp DNA blood Mini Kit, according to manufacturer's instruction (Qiagen GmbH, Hilden, Germany).Genotyping of *IL-18* -137G/C and -607C/A polymorphism was performed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primer sequences, restriction enzymes, restriction digest products of *IL-18* -137G/C and -607C/A were shown in **Table 1**. The PCR amplification was carried out with initial denaturation at 94°C for 5 minutes, and then 35 cycles of denaturation at 94°C for 35 seconds, annealing at 62°C for 48 seconds and extension for 72°C for 55 seconds, and a finally step of extension for 72°C for 5 minutes. The amplification products were mixture with 1 μL 10 × loading Buffer, were analyzed using electrophoresis on a 2% agarose gel, and were observed under ultraviolet light and compared with 100 bp DNA Marker (**Figures 1** and **2**).

Statistical analysis

Categorical variables were expressed as percentages of total, and or continuous variables were shown as mean ± SD. Student's or Pearson chi-square test were used to compare the differences between groups. Genotypes of *IL-18* -137G/C and -607C/A were tested for departures from Hardy-Weinberg equilibrium (HWE) in the control population. Logistic regression analysis was performed in order to determine the odds ratios (OR) and 95% confidence intervals (95% CI) associated with the cervical cancer risk, taking the control as the reference

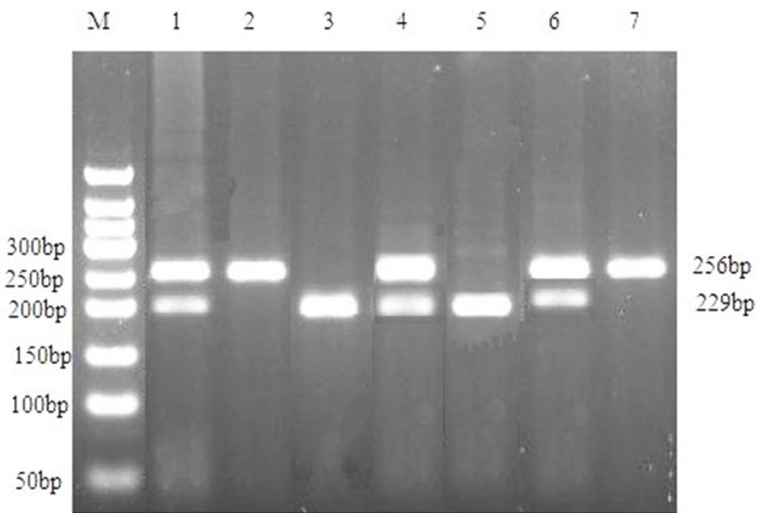

Figure 1. Agarose gel electrophoresis images for *IL-18* -137G/C. 1, 4 and 6 lanes: GC genotype; 2 and 7 lanes: CC genotype: 3 and 5 lanes: GG genotype.

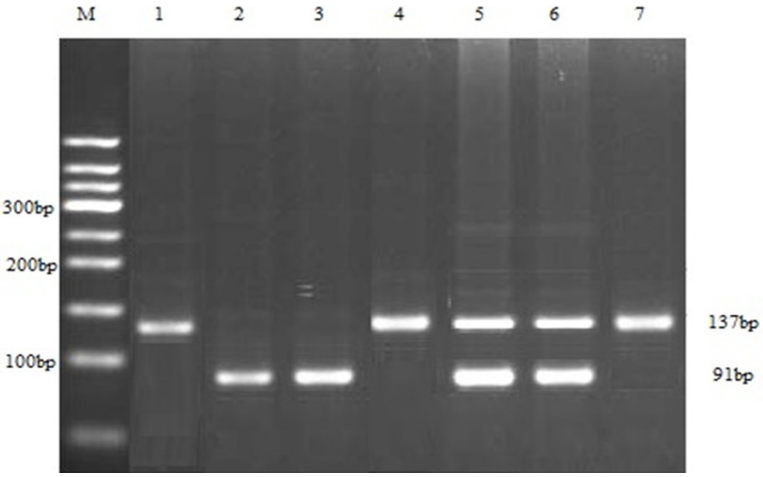

Figure 2. Agarose gel electrophoresis images for *IL-18* -607C/A. 1, 4 and 7 lanes: CC genotype; 2 and 3 lanes: AA genotype; 5 and 6 lanes: CA genotype.

group. Statistical significance was set at P<0.05.

Results

The demographic and clinical variables of investigated study subjects were shown in **Table 2**. Using chi-square or Student's tests, there were significant differences between diabetic nephropathy patients and controls in respect to BMI (t = 3.88, P<0.001), hypertension (χ^2 = 6.82, P = 0.009), glucose (t = 21.29, P<0.001), HbA1c (t = 20.57, P<0.001), triglyceride (t = 4.59, P<0.001), total cholesterol (t = 4.53, P<0.001), low-density lipoprotein (t = 8.37, P<0.001) and creatinine (t = 66.03, P<0.001). However, no significant differences were observed between diabetic nephropathy patients and controls in terms of age (t = 0.86, P = 0.196), gender (chi-square = 0.09, P = 0.780) and high-density lipoprotein (t = 1.06, P = 0.144).

The genotype distributions of *IL-18* -137G/C and -607C/A of the two investigated groups were shown in **Table 3**. Genotype distributions of *IL-18* -137G/C (chi-square = 0.14, P = 0.705) and -607C/A (chi-square = 2.12, P = 0.145) were not departure from Hardy-Weinberg equilibrium (**Table 2**). The GG, GC and CC genotypes of *IL-18* -137G/C were significant differences between diabetic nephropathy patients and controls (chi-square = 16.12, P< 0.001), and the G and C alleles of *IL-18* -137G/C showed also significant differences between the two groups (chi-square = 11.28, P<0.001). However, no significant differences were observed in the genotype (chi-square = 1.77, P = 0.412) and allele (chi-square = 1.14, P = 0.285) distributions of *IL-18* -607C/A between the two investigated groups.

As determined by multiple logistic regression analysis, individuals carrying GC genotype of *IL-18* -137G/C were correlated with an elevated risk of diabetic nephropathy in comparison with the GG genotype, and the crude and adjusted ORs were 2.31 (1.49-3.56) and 2.54 (1.63-4.11), respectively (**Table 4**). Moreover, the C allele of *IL-18* -137G/C was associated with risk of developing diabetic nephropathy in compari-

Table 2. Demographic characteristics of diabetic nephropathy patients and controls

Variables	Patients N = 155	%	Controls N = 320	%	χ^2 test or *t* test	*P* value
Age, years	58.53±9.12		57.78±8.85		0.86	0.196
Gender						
Female	58	37.42	124	38.75		
Male	97	62.58	196	61.25	0.09	0.780
BMI, kg/m^2	25.25±3.22		24.05±3.13		3.88	<0.001
Hypertension						
No	84	54.19	213	66.56		
Yes	71	45.81	107	33.44	6.82	0.009
Glucose, mml/L	11.54±3.74		6.21±1.45		21.29	<0.001
HbA1c, %	9.13±2.53		5.03±1.75		20.57	<0.001
Triglyceride, mmol/L	1.65±0.22		1.56±0.19		4.59	<0.001
Total cholesterol, mmol/L	5.15±1.05		4.72±0.93		4.53	<0.001
High-density lipoprotein, mmol/L	1.35±0.52		1.30±0.46		1.06	0.144
Low-density lipoprotein, mmol/L	3.97±1.50		2.78±1.43		8.37	<0.001
Creatinine, mmol/L	146.76±12.14		74.12±10.78		66.03	<0.001
Duration of diabetes, years	11.22±4.17					

Table 3. Genotype distributions of *IL-18* -137G/C and -607C/A between the two groups

IL-18	Patients N = 155	%	Controls N = 320	%	χ^2 value	*P* value	χ^2 value for HWE	*P* value for HWE
-137G/C								
GG	88	56.77	238	74.38				
GC	64	41.29	75	23.44				
CC	3	1.94	7	2.19	16.12	<0.001	0.14	0.705
Allele								
G	240	77.42	551	86.09				
C	70	22.58	89	13.91	11.28	<0.001		
-607C/A								
CC	40	25.81	100	31.25				
CA	73	47.10	146	45.63				
AA	42	27.10	74	23.13	1.77	0.412	2.12	0.145
Allele								
C	153	49.35	346	54.06				
A	157	50.65	294	45.94	1.14	0.285		

son with G allele, and the crude and adjusted ORs were 1.81 (1.25-2.59) and 2.16 (1.36-2.85), respectively. However, *IL-18* -607C/A polymorphism was not associated with the risk of developing diabetic nephropathy.

Discussion

Diabetic nephropathy is the most common complication of diabetes mellitus. The pathological processes of diabetic nephropathy include kidney hypertrophia, thickened basement membrane of glomerular and renal tubules, and gradual accumulation of extracullular matrix of glomerular and tubulointerstitial [10, 11]. In the present study, we investigated the association between *IL-18* -137G/C and -607C/A genetic polymorphisms and risk of diabetic nephropathy, and we observed that GC genotype and C allele *IL-18* -137G/C contributed to the risk of diabetes mellitus in the investigated population.

Table 4. Association between *IL-18* -137G/C and -607C/A polymorphisms and risk of diabetic nephropathy

IL-18	Patients	%	Controls	%	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
-137G/C								
GG	88	56.77	238	74.38	1.0 (Ref.)	-	1.0 (Ref.)	-
GC	64	41.29	75	23.44	2.31 (1.49-3.56)	<0.001	2.54 (1.63-4.11)	<0.001
CC	3	1.94	7	2.19	1.16 (0.19-5.21)	0.83	1.57 (0.32-6.67)	0.072
Allele								
G	240	77.42	551	86.09	1.0 (Ref.)	-	1.0 (Ref.)	-
C	70	22.58	89	13.91	1.81 (1.25-2.59)	<0.001	2.16 (1.36-2.85)	<0.001
-607C/A								
CC	40	25.81	100	31.25	1.0 (Ref.)	-	1.0 (Ref.)	-
CA	73	47.10	146	45.63	1.25 (0.77-2.04)	0.34	1.36 (0.79-2.31)	0.25
AA	42	27.10	74	23.13	1.42 (0.81-2.49)	0.19	1.53 (0.84-2.63)	0.24
Allele								
C	153	49.35	346	54.06	1.0 (Ref.)	-	1.0 (Ref.)	-
A	157	50.65	294	45.94	1.21 (0.91-1.60)	0.17	1.35 (0.94-1.72)	0.28

IL-18 is a pleiotropic cytokine, and is mainly produced by the activation of mononuclear macrophages. IL-18 induces the production of IFN-γ, and participates into pathological process of various diseases [12]. Previous experimental studies have revealed that IL-18 may contribute to the development of diabetic nephropathy in diabetic mice models. IL-18 could promote the proliferation of glomerular mesangial cells and release prostaglandin through influencing the mitosis of glomerular mesangial cells, and thus promote glomerular high filtration and cause the pathological changes of glomerular capillary [13]. IL-18 could release collagen ase and extracellular protease through stimulation of renal interstitium cell near the glomerular capillary [14]. The previous studies have indicated that IL-18 could cause the development of diabetic nephropathy.

The mutation includes the transformation of a single base by transversion, insertion, or deletions, and the SNP is thought to result in susceptibility to human diseases [15-17]. Three polymorphic sites, -137, -607 and -656, are located at promoter region of exon 1 in IL-18, and they could influence the expression and secretion of IL-18 and expression of IFN-γ [7].

Previous studies have reported the association of IL-18 polymorphisms with the development of diabetes or nephropathy, but the results are conflicting [18-23]. Jung et al. carried out a study with 146 normal individuals and 69 IgA nephropathy and 44 thin glomerular basement membrane disease patients, and have revealed that *IL-18* -607C/A polymorphism could increase the risk of IgA nephropathy and thin glomerular basement membrane disease [20]. Kariž and Petrovič performed a study with 495 Caucasians with type 2 diabetes, and have revealed that *IL-18* -137G/C could not affect the development of myocardial infarction in type 2 diabetes [21]. Tavares et al. have indicated that *IL-18* -137G/C polymorphism is associated with susceptibility to type 1 diabetes [23]. Lee et al. have carried out a meta-analysis with ten studies, and have reported that *IL-18* -607C/A polymorphism may be correlated with susceptibility to type 1 diabetes [19]. However, Pan et al. have reported that *IL-18* -607C/A polymorphism is not associated with the development of type 1 diabetes [22].

Currently, only one study reported the association between IL-18 polymorphism and risk of cardiovascular diseases in diabetic nephropathy patients [8]. Szeto et al. carried out a study with 220 patients, and have reported that *IL-18* -137G/C polymorphism is correlated with the cardiovascular mortality in patients with diabetic nephropathy [8]. In our study, we firstly reported a significant association between

IL-18 -137G/C polymorphism and risk of diabetic nephropathy. Further studies with large sample size are greatly needed to confirm the results of our findings.

In conclusion, our study suggests that *IL-18* -137G/C is associated with development of diabetic nephropathy in the Chinese population. Further studies large-scale studies should be conducted to gain better insight into the impact of *IL-18* -137G/C and -607C/A polymorphism on the risk of diabetic nephropathy.

Acknowledgements

This work was supported by the key projects from Henan Provincial Committee of Education.

Disclosure of conflict of interest

None.

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