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Original Article

Association of two common SNPs in *NLRP3* with risk of type 2 diabetes mellitus and their interaction with environmental factors

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Abstract: Type 2 diabetes mellitus, a chronic and complex disease. *NLRP3* inflammasome plays an important role in the obesity induced inflammation and insulin resistance, and polymorphisms of *NLRP3* may contribute to the development of type 2 diabetes mellitus. Here, we carried out a study to investigate the role of two SNPs of *NLRP3* (rs10754558 and rs4612666) in the risk of type 2 diabetes mellitus in a Chinese population. This study comprised of 286 patients with type 2 diabetes mellitus and 306 healthy control subjects. Genotyping of *NLRP3* rs10754558 and rs4612666 was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Using unconditional logistic regression analysis, we observed that homozygous *NLRP3* rs10754558 CC carriers demonstrated a significantly higher risk of developing type 2 diabetes mellitus in comparison to homozygous GG carriers, with an adjusted OR (95% CI) of 2.38 (1.53-3.97). Moreover, the C allele of *NLRP3* rs10754558 was associated with an elevated risk of *NLRP3* rs4612666 when compared to G allele (OR = 1.51, 95% CI = 1.18-1.91). However, we observed no significant relationship between *NLRP3* rs4612666 genetic polymorphisms and type 2 diabetes mellitus risk in the Chinese population. In summary, our study has shown that the polymorphism of *NLRP3* rs10754558 is associated with high risk of type 2 diabetes mellitus.

Keywords: *NLRP3,* rs10754558, rs4612666, polymorphism, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus, a chronic and complex disease, has become a challenging problem which severely threatens public health. Type 2 diabetes mellitus is associated with high morbidity worldwide, and the number of individuals in China suffering from type 2 diabetes mellitus shows a tendency to increase [1]. It is estimated that there are 92,400,000 adults suffering from type 2 diabetes mellitus and 148,200,000 individuals suffering from prediabetes [1]. Even though improvements in early detection have reduced the rates of diabetic complications in recent years, prevention of type 2 diabetes mellitus is always a main public health concern. The pathogenesis of type 2 diabetes mellitus occurs over a long period of time, involves many environmental and lifestyle factors, such as high fat dietary, high cholesterol dietary, obesity or overweight and lack of activity, as well as hypertension [2, 3]. Additionally, genome-wide association studies have implicated many genetic loci in the onset, prognosis, or severity of type 2 diabetes mellitus [4, 5].

NLRP3 belongs to the family of NOD-like receptor (NLP) proteins, and it locates on chromosome 1q44 and is 30 kilobases (kb) in length with 9 exons and 8 introns. *NLRP3* could active caspase-1, and participate in synthesis, processing and production of IL-1β. IL-1β has an important role in the development of chronic inflammatory diseases [6-8]. A current study has reported that the *NLRP3* inflammasome plays an important role in the obesity induced inflammation and insulin resistance [9]. Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by a single nucleotide variation, and the frequency of genetic polymorphisms is at least 1% in a population. The mutations include the transformation of a single base by transversion, insertion,

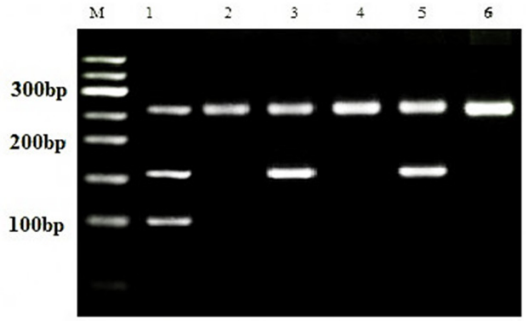

Figure 1. Agarose gel electrophoresis images for *NLRP3* rs10754558. 1 lane: GC genotype; 2, 4 and 6 lanes: GG genotype; 3 and 5 lane: CC genotype.

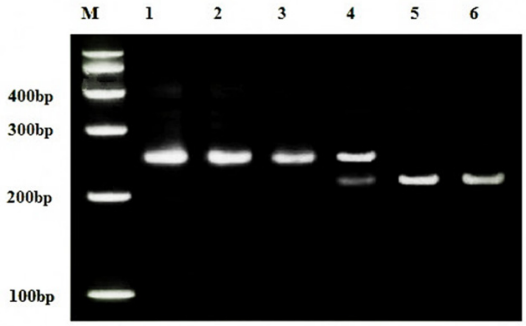

Figure 2. Agarose gel electrophoresis images for *NLRP3* rs4612666. 1-3 lanes: TT genotype; 4 lane: TC genotype; 5 and 6 lane: CC genotype.

or deletion, and SNPs are thought to affect susceptibility to human diseases. Genomic polymorphisms of *NLRP3* could influence the structure, expression and quantity of *NLRP3*, and ultimately affect the function of genes. Currently, a few studies have reported the relationship between *NLRP3* genetic polymorphisms and development of type 2 diabetes mellitus, but the results are inconsistent [10, 11]. However, no study reports the interaction of *NLRP3* genetic polymorphisms and environmental factors in the risk of type 2 diabetes. Therefore, in our study, we carried out a study to investigate the role of two common SNPs of *NLRP3* (rs10754558 and rs4612666) in the risk of type 2 diabetes mellitus and their interaction with environmental factors in a Chinese population.

Materials and methods

Subjects

This study comprises of 286 patients with type 2 diabetes mellitus and 306 healthy control subjects. The patients with type 2 diabetes mellitus were consecutively recruited from the First Affiliated Hospital of Xinxiang Medical University between February 2013 and May 2015. The type 2 diabetes mellitus was diagnosed according to the criteria from the WHO-IDF criteria (WHO-IDF, 2014). Patients who had a history of type I diabetes mellitus, malignant tumors, acute or chronic infection disease, or other endocrine diseases except for type 2 diabetes mellitus, as well as end-stage liver and kidney diseases were excluded from this study.

The control subjects were recruited from individuals who visited the outpatient clinics, and all the control subjects received health examination and were confirmed to have no history of type 2 diabetes mellitus, type I diabetes mellitus, malignant tumors, acute or chronic infection disease, or endocrine diseases as well as end-stage liver and kidney diseases.

The demographic and lifestyle data of type 2 diabetes mellitus patients and control subjects, including age, gender, tobacco smoking, alcohol consumption, hypertension, body mass index (BMI) and family history of type 2 diabetes mellitus, were collected from a structured questionnaire investigated by face-to-face investigation with trained nurses. The clinical data, including duration of diabetes, fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c), were included from our study. Informed consent was obtained from all participants in the study. All methods mentioned below were approved by the ethics committee of the First Affiliated Hospital of Xinxiang Medical University and were carried out in accordance with the approved guidelines.

DNA extraction and genotyping

Five mL peripheral blood sample was obtained from each study subject, which was stored using ethylene diamine tetra-acetic acid (EDTA)-coated tubes. DNA was extracted from the blood samples using QIAamp DNA Blood Mini Kit (QIAGEN, USA) following the manufacturer's recommendation. Genotyping of *NLRP3* rs10754558 and rs4612666 was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primer sequences for *NLRP3* rs10754558 were 5'-TGCTTAAGGCCATTAATTGTG-3' and 5'-CTCC-

Table 1. Demographic, lifestyle and clinical characteristics of the study subjects

Variables	Patients N = 286	%	Controls N = 306	%	χ^2-test or t-test	*P* value
Age, years		53.56±8.45		53.14±9.53	0.57	0.29
Gender						
Females	99	34.62	139	45.42		
Males	187	65.38	167	54.58	7.19	0.01
Tobacco smoking						
No	161	56.29	191	62.42		
Yes	125	43.71	115	37.58	2.30	0.13
Alcohol consumption						
No	178	62.24	207	67.65		
Yes	108	37.76	99	32.35	1.90	0.17
Hypertension						
No	164	57.34	237	77.45		
Yes	122	42.66	69	22.55	27.35	< 0.001
BMI, kg/m^2		27.85±9.43		24.62±10.64	3.90	< 0.001
Family history of type 2 diabetes mellitus						
No	230	80.42	286	93.46		
Yes	56	19.58	20	6.54	22.48	< 0.001
FPG, mmol/dL		9.14±2.85		4.64±3.12	18.28	< 0.001
Fasting insulin, mmol/dL		60.43±18.31		48.72±16.44	8.20	< 0.001
TC, mmol/dL		5.36±1.17		4.55±0.95	9.27	< 0.001
TG, mmol/dL		2.07±1.16		1.05±0.48	14.14	< 0.001
LDL-c, mmol/dL		2.83±0.85		2.87±0.76	0.60	0.27
HDL-c, mmol/dL		1.27±0.35		1.62±0.37	11.81	< 0.001

BMI: body mass index; FPG: fasting plasma glucose; TC: total cholesterol; TG: triglyceride; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol.

ACCATGGACAAGGAAG-3', respectively. The primer sequences for NLRP3 rs4612666 were 5'-CAGGACAATGACAGCATCGGGTGTTGAT-3' and 5'-GCTGCCATAAAATTTCAACATAA-3', respectively. The restriction enzymes for *NLRP3* rs10754558 and rs4612666 were BpiI and MboI, respectively. PCR was performed in a 15 μl reaction mixture containing 5 μl ddH_2O, 1 μl DNA template, 0.5 μl of each primer, 1.0 unit of Dream Tag™Green PCR Mix (2X) and 10 × Green buffer. The PCR condition was set at: initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56.1°C for 30 seconds, and extension at 72°C for 60 seconds; and a final extension at 72°C for 7 minutes.

PCR products were digested by *Bpi*I and *Mbo*I to distinguish the *NLRP3* rs10754558 and rs4612666, respectively. The product lengths of PCR reaction for rs10754558 and rs4612666 were 260 bp and 261 bp, respectively. For rs10754558, the GG genotype was digested into 260 bp fragments, the GC genotype was digested into 260 bp, 158 bp and 102 bp fragments, and the CC genotype was digested into158 bp and 102 bp fragments (**Figure 1**). For rs4612666, the TT genotype was digested into 261 bp fragments, the TC genotype was digested into 261 bp, 236 bp and 25 bp fragments, and the CC genotype was digested into 236 bp and 25 bp fragments (**Figure 2**). The PCR products were analyzed by electrophoresis on a 2% agarose gel and stained with ethidium bromide. The DNA bands were visualized under UV light.

Statistical analysis

The demographic and clinical characteristics, as well as *NLRP3* rs10754558 and rs4612666 genotype frequencies between type 2 diabetes mellitus patients and healthy control subjects were compared using chi-squared (χ^2) tests or

Table 2. Genotype frequencies of *NLRP3* rs10754558 and rs4612666 between the study groups

NLRP3	Patients N = 286	%	Controls N = 306	%	χ^2-test	*P* value	HWE		MAF	
							Patients	Controls	Controls	Database
rs10754558										
GG	90	31.47	125	40.85						
GC	131	45.80	143	46.73						
CC	65	22.73	38	12.42	12.64	0.002	0.19	0.77	0.3578	0.3464
rs4612666										
TT	75	26.22	83	27.12						
TC	120	41.96	137	44.77						
CC	91	31.82	86	28.10	0.99	0.61	0.01	0.07	0.5049	0.4109

HWE: Hardy-Weinberg Equilibrium; MAF: minor allele frequency.

Student's *t*-test. The goodness-of-fit χ^2-test was performed to determine whether the genotype frequencies at *NLRP3* rs10754558 and rs4612666 were deviation from expected allele frequencies and was in agreement with the Hardy-Weinberg Equilibrium (HWE). The minor allele frequencies (MAFs) of *NLRP3* rs10754558 and rs4612666 were calculated to analyze whether they are similar with those in database from National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/snp). Univariate and multivariate logistic regression analyses were taken to analyze the association between *NLRP3* rs10754558 and rs4612666 polymorphisms and susceptibility to type 2 diabetes mellitus. The results were determined using odd ratio (OR) along with 95% confidence interval (CI), and adjusted for potential confounding factors. The interaction of *NLRP3* rs10754558 and rs4612666 polymorphisms was done by Spearman interaction analysis. All the analyses were carried out using SPSS version 20.0 (Armonk, NY: IBM Corp, USA). A *P*-value < 0.05 at 95% confidence interval (CI) was taken as statistically significant.

Results

The demographic, lifestyle and clinical characteristics of the study subjects are summarized in **Table 1**. The mean ages of included type 2 diabetes mellitus patients and control subjects were 53.56±8.45 and 53.14±9.53 years, respectively. There were 99 (34.62%) females and 187 (65.38%) males in the type 2 diabetes mellitus patients, and there were 139 (45.42%) females and 167 (54.58%) males in control subjects. In comparison to healthy controls, type 2 diabetes mellitus patients were more likely to be males (t = 0.57, P = 0.29), suffer from hypertension (χ^2 = 27.35, P < 0.001), have family history of type 2 diabetes mellitus (χ^2 = 22.48, P < 0.001), and have higher levels of BMI (t = 3.90, P < 0.001), FPG (t = 18.24, P < 0.001), TC (t = 9.27, P < 0.001), TG (t = 14.14, P < 0.001) and lower levels of HDL-c (t = 11.81, P < 0.001).

We further analyzed the genotype distributions of *NLRP3* rs10754558 and rs4612666 between type 2 diabetes mellitus patients and control subjects (**Table 2**). The genotype distributions of *NLRP3* rs10754558 significantly differed between the two study groups (χ^2 = 12.64, P = 0.002), whereas the type 2 diabetes mellitus patients and control subjects were comparable in regard to genotype frequencies of NLRP3 rs4612666 (χ^2 = 1.12, P = 0.57). The *NLRP3* rs10754558 genotype frequencies were in agreement with the HWE in both patients and controls (*P* values of HWE were 0.19 and 0.77 for patients and controls, respectively), whereas the NLRP3 rs4612666 was not (*P* values of HWE were 0.01 and 0.07 for patients and controls, respectively). The MAF values of NLRP3 rs10754558 and rs4612666 in the controls were similar with those in database from National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/snp).

We then analyzed the relationship between *NLRP3* rs10754558 and rs4612666 genetic polymorphisms and development of type 2 diabetes mellitus (**Table 3**). Using unconditional logistic regression analysis, we observed that homozygous *NLRP3* rs10754558 CC carriers

Table 3. Relationship between *NLRP3* rs10754558 and rs4612666 genetic polymorphisms and development of type 2 diabetes mellitus

NLRP3	Patients N = 286	%	Controls N = 306	%	OR (95% CI)[1]	*P* value
rs10754558						
GG	90	31.47	125	40.85	1.0 (Ref.)	-
GC	131	45.8	143	46.73	1.27 (0.87-1.85)	0.19
CC	65	22.73	38	12.42	2.38 (1.53-3.97)	0.0004
Allele						
G	311	54.37	393	64.215	1.0 (Ref.)	-
C	261	45.63	219	35.785	1.51 (1.18-1.91)	0.0006
rs4612666						
TT	75	26.22	83	27.12	1.0 (Ref.)	-
TC	120	41.96	137	44.77	0.97 (0.64-1.47)	0.88
CC	91	31.82	86	28.1	1.17 (0.75-1.84)	0.47
Allele						
T	270	47.2	303	49.51	1.0 (Ref.)	-
C	302	52.8	309	50.49	1.10 (0.87-1.39)	0.43

[1]Adjusted for gender, hypertension, BMI, family history of type 2 diabetes mellitus, FPG, fasting insulin, TC, TG and HDL-c. OR: odds ratio; 95% CI: confidence interval.

Table 4. Interaction of *NLRP3* rs10754558 and rs4612666 genetic polymorphisms and demographic, lifestyle and clinical characteristics

Variables	rs10754558		rs4612666	
	Correlation coefficient value	*P* value	Correlation coefficient value	*P* value
Age	0.011	0.54	0.009	0.65
Male	0.008	0.63	0.017	0.42
Tobacco smoking	0.014	0.50	0.012	0.51
Alcohol consumption	0.020	0.34	0.015	0.44
Hypertension	0.012	0.51	0.022	0.39
BMI	0.016	0.47	0.017	0.40
Family history of type 2 diabetes	0.054	0.02	0.024	0.35
FPG	0.017	0.41	0.011	0.56
Fasting insulin	0.022	0.38	0.018	0.37
TC	0.019	0.35	0.013	0.45
TG	0.008	0.65	0.022	0.38
LDL-c	0.010	0.56	0.014	0.46
HDL-c	0.015	0.42	0.024	0.32

demonstrated a significantly higher risk of developing type 2 diabetes mellitus in comparison to homozygous GG carriers, with an adjusted OR (95% CI) of 2.38 (1.53-3.97). Moreover, the C allele of *NLRP3* rs10754558 was associated with an elevated risk of *NLRP3* rs4612666 when compared to G allele (OR = 1.51, 95% CI = 1.18-1.91). However, we observed no significant relationship between *NLRP3* rs4612666 genetic polymorphisms and type 2 diabetes mellitus risk in the Chinese population.

Subgroup analyses were stratified by demographic, lifestyle and clinical factors were done (**Table 4**), and we revealed a significant interaction between *NLRP3* rs10754558 and rs4612666 genetic polymorphisms and family history of type 2 diabetes mellitus in the risk of type 2 diabetes mellitus (Correlation coefficient value = 0.054, P = 0.02).

Discussion

Type 2 diabetes mellitus is a severe public health problem in China. It is estimated that the incidence of type 2 diabetes mellitus is about 9.7%, and there are 92 million patients suffered from this disease. So far, the identified factors correlated with type 2 diabetes mellitus are environment, ethnicity, family history and genetic mutation. Current studies have revealed that occurrence of type 2 diabetes mellitus is closely associated with insulin resistance and chronic inflammation [12, 13]. Type 2 diabetes mellitus may be mediated by cytokines inflammation, and is a kind of innate immunity disease [14, 15]. Previous study has reported that IL-1β can mediate glucose toxicity, destroy the insulin-producing cells of the pancreas and is associated with the occurrence of insulin resistance [16]. The IL-1β has been become a recognized factor for to damage pancreatic β cell and inflammatory cyto-

kines [17]. Caspase-1 of *NLRP3* could split precursor intracellular IL-1β at 116 aspartic acid and form activated mature IL-1β, and produce various extracellular inflammatory response [18]. A current study has reported that the *NLRP3* inflammasome plays an important role in the obesity induced inflammation and insulin resistance [9]. In our study, we investigated the relationship between *NLRP3* rs10754558 and rs4612666 genetic polymorphisms and development of type 2 diabetes mellitus, and we revealed that *NLRP3* rs10754558 genetic variation was associated with risk of developing type 2 diabetes mellitus.

The *NLRP3* gene is located in 1q44, and this gene is reported to be associated with many immune inflammatory diseases, such as primary gout, ischemic stroke, blood pressure, recurrent aphthous stomatitis and juvenile spondyloarthritis [19-25]. Deng et al. carried a study in a Chinese population, and reported that rs3806268 in *NLRP3* gene was associated with the risk of primary gout [19]. Zhu et al. reported that rs10754558 could influence mRNA level of NLRP3 and ischemic stroke risk [20]. Zhang et al. revealed that *NLRP3* rs35829419 polymorphism was associated with a markedly increase susceptibility to various diseases in human, such as leprosy, colorectal cancer, rheumatoid arthritis and so on [21]. Kunnas et al. discovered that *NLRP3* rs7512998 was correlated with systolic and diastolic blood pressure in a Finnish population [22]. Bidoki et al. conducted a study in an Iranian population, and reported that *NLRP3* rs3806265 TT genotype was associated with the susceptibility to recurrent aphthous stomatitis [24]. Perica et al. reported that the *NLRP3* 35829419 polymorphism was not associated with development of juvenile spondyloarthritis [25].

For the relationship between *NLRP3* genetic polymorphisms and development of type 2 diabetes mellitus, only two studies reported their association [10, 11]. Wang et al. carried out a case-control study with 385 patients with type 2 diabetes mellitus and 401 control subjects in China, and reported that *NLRP3* rs10754558 polymorphism contributes to type 2 diabetes mellitus risk, but that rs7512998 and rs12137901 variants were not correlated with susceptibility to this disease [10]. Zheng et al. done a study with 952 type 2 diabetes mellitus patients and 871 control subjects, and discovered that *NLRP3* rs10754558 polymorphism was associated with insulin resistance and elevated risk of type 2 diabetes mellitus in a Chinese population [11]. In our study, we also discovered that the CC genotype and C allele of *NLRP3* rs10754558 variant was correlated with the development of type 2 diabetes mellitus when compared with the wide-type genotype. Further studies are greatly needed to confirm our findings.

However, our study had some limitations. First, the selection bias might not be avoided since the study subjects were selected from only one hospital. Second, possibility of gene-gene or SNP-SNP interactions, or linkage disequilibrium between polymorphism may have a role in the pathogenesis of type 2 diabetes mellitus. Third, our study had limited statistical power possibly due to a small sample size. Therefore, further molecular genetic studies should be performed in a large population sample in order to identify the mechanisms role of *NLRP3* genetic polymorphisms in the development of type 2 diabetes mellitus.

In summary, our study has shown that the polymorphism of *NLRP3* rs10754558 is associated with heavy risk of type 2 diabetes mellitus. Future study should include larger sample size to provide a greater view for the relationship between *NLRP3* genetic polymorphisms and development of type 2 diabetes mellitus risk in different populations.

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Disclosure of conflict of interest

None.

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References

[1] Yang SH, Dou KF, Song WJ. Prevalence of diabetes among men and women in China. N Engl J Med 2010; 362: 2425-6.

[2] Pearson ER. Dissecting the Etiology of Type 2 Diabetes in the Pima Indian Population. Diabetes 2015; 64: 3993-5.
[3] Mrozikiewicz-Rakowska B, Maroszek P, Nehring P, Sobczyk-Kopciol A, Krzyzewska M, Kaszuba AM, Lukawska M, Chojnowska N, Kozka M, Bujalska-Zadrozny M, Ploski R, Krzymien J, Czupryniak L. Genetic and environmental predictors of chronic kidney disease in patients with type 2 diabetes and diabetic foot ulcer: a pilot study. J Physiol Pharmacol 2015; 66: 751-61.
[4] Burdon KP, Fogarty RD, Shen W, Abhary S, Kaidonis G, Appukuttan B, Hewitt AW, Sharma S, Daniell M, Essex RW, Chang JH, Klebe S, Lake SR, Pal B, Jenkins A, Govindarjan G, Sundaresan P, Lamoureux EL, Ramasamy K, Pefkianaki M, Hykin PG, Petrovsky N, Brown MA, Gillies MC, Craig JE. Genome-wide association study for sight-threatening diabetic retinopathy reveals association with genetic variation near the GRB2 gene. Diabetologia 2015; 58: 2288-97.
[5] Go MJ, Hwang JY, Park TJ, Kim YJ, Oh JH, Kim YJ, Han BG, Kim BJ. Genome-wide association study identifies two novel Loci with sex-specific effects for type 2 diabetes mellitus and glycemic traits in a korean population. Diabetes Metab J 2014; 38: 375-87.
[6] Fernandes MT, Fernandes KB, Marquez AS, Cólus IM, Souza MF, Santos JP, Poli-Frederico RC. Association of interleukin-6 gene polymorphism (rs1800796) with severity and functional status of osteoarthritis in elderly individuals. Cytokine 2015; 75: 316-20.
[7] Cui L, Gao Y, Xie Y, Wang Y, Cai Y, Shao X, Ma X, Li Y, Ma G, Liu G, Cheng W, Liu Y, Liu T, Pan Q, Tao H, Liu Z, Zhao B, Shao Y, Li K. An ADAM10 promoter polymorphism is a functional variant in severe sepsis patients and confers susceptibility to the development of sepsis. Crit Care 2015; 19: 73.
[8] Opipari A, Franchi L. Role of inflammasomes in intestinal inflammation and Crohn's disease. Inflamm Bowel Dis 2015; 21: 173-81.
[9] Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011; 17: 179-88.
[10] Wang S, Fang F, Jin WB, Wang X, Zheng XS. Investigation into the association between NLRP3 gene polymorphisms and susceptibility to type 2 diabetes mellitus. Genet Mol Res 2015; 14: 17447-52.
[11] Zheng Y, Zhang D, Zhang L, Fu M, Zeng Y, Russell R. Variants of NLRP3 gene are associated with insulin resistance in Chinese Han population with type-2 diabetes. Gene 2013; 530151-4.
[12] Tracy RP. Diabetes and atherothrombotic disease: linked through inflammation? Semin Vasc Med 2002; 2: 67-73.
[13] Ferroni P, Basili S, Falco A, Davì G. Inflammation, insulin resistance, and obesity. Curr Atheroscler Rep 2004; 6: 424-31.
[14] Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N, Halban PA, Donath MY. Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest 2002; 110: 851-60.
[15] Leinonen E, Hurt-Camejo E, Wiklund O, Hultén LM, Hiukka A, Taskinen MR. Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. Atherosclerosis 2003; 166: 387-94.
[16] Maedler K, Dharmadhikari G, Schumann DM, Størling J. Interleukin-1 beta targeted therapy for type 2 diabetes. Expert Opin Biol Ther 2009; 9: 1177-88.
[17] Liu D, Pavlovic D, Chen MC, Flodström M, Sandler S, Eizirik DL. Cytokines induce apoptosis in beta-cells isolated from mice lacking the inducible isoform of nitric oxide synthase (iNOS-/-). Diabetes 2000; 49: 1116-22.
[18] Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: master switches of inflammation. Cell Death Differ 2007; 14: 10-22.
[19] Deng J, Lin W, Chen Y, Wang X, Yin Z, Yao C, Liu T, Lv Y. rs3806268 of NLRP3 gene polymorphism is associated with the development of primary gout. Int J Clin Exp Pathol 2015; 8: 13747-52.
[20] Zhu Z, Yan J, Geng C, Wang D, Li C, Feng S, Wang H. A Polymorphism Within the 3'UTR of NLRP3 is Associated with Susceptibility for Ischemic Stroke in Chinese Population. Cell Mol Neurobiol 2016; 36: 981-8.
[21] Zhang Q, Fan HW, Zhang JZ, Wang YM, Xing HJ. NLRP3 rs35829419 polymorphism is associated with increased susceptibility to multiple diseases in humans. Genet Mol Res 2015; 14: 13968-80.
[22] Kunnas T, Määttä K, Nikkari ST. NLR family pyrin domain containing 3 (NLRP3) inflammasome gene polymorphism rs7512998 (C > T) predicts aging-related increase of blood pressure, the TAMRISK study. Immun Ageing 2015; 12: 19.
[23] Klen J, Goričar K, Janež A, Dolžan V. NLRP3 Inflammasome Polymorphism and Macro-

vascular Complications in Type 2 Diabetes Patients. J Diabetes Res 2015; 2015: 616747.

[24] Bidoki AZ, Harsini S, Sadr M, Soltani S, Mohammadzadeh M, Najafi S, Rezaei N. NLRP3 gene polymorphisms in Iranian patients with recurrent aphthous stomatitis. J Oral Pathol Med 2016; 45: 136-40.

[25] Perica M, Vidović M, Lamot L, Bukovac LT, Kapitanović S, Perić M, Barbić J, Harjaček M. Single nucleotide polymorphism of toll-like receptor 4 (TLR4) is associated with juvenile spondyloarthritis in Croatian population. Clin Rheumatol 2015; 34: 2079-86.