Genetic association of Toll-like receptor 4 gene and coronary artery disease in a Chinese Han population

Liting Zhou¹, Dongchun Zheng¹, Shuyue Wang², Jian Zhu¹, Yiyang Jia¹, Di Sun¹, Jin Xu¹, Qi Wang¹, Huaiji Chen¹, Feng Xu¹, Bo Li³* and Lin Ye¹*

Abstract

Purpose: Toll-like receptor 4 (TLR4) is known to be involved in innate immunity and inflammatory responses that play important roles in the pathogenesis of coronary artery disease (CAD). But the relationship between TLR4 gene and CAD has yet to be investigated. The present study aimed to evaluate the association of TLR4 gene polymorphisms with CAD susceptibility in a Chinese Han population.

Methods: A total of 1094 subjects (577 unrelated patients with CAD and 517 controls) were recruited between 2008 and 2012. Three tag SNPs (rs1927907, rs1927911 and rs11536889) present in the TLR4 gene were genotyped using Sequenom Mass-ARRAY system.

Results: The genotypic distributions of the three SNPs were not deviate from Hardy–Weinberg equilibrium. There was no significant difference in distributions of allelic frequencies of each SNPs between healthy controls and CAD patients (P > 0.05). Genotype frequencies of TLR4 gene did not show any statistically significant difference between the two groups in co-dominant, dominant or recessive genetic models (P > 0.05). The frequency of haplotypes in the case group was similar to that in the control group (P > 0.05).

Conclusion: TLR4 gene do not relate to genetic susceptibility of CAD in the Chinese Han population.

Keywords: Coronary artery disease, Gene polymorphism, Toll-like receptor 4

Background

Coronary artery disease (CAD) is the leading cause of morbidity and mortality worldwide (Roger et al. 2012). As a complex disease, the main pathological features of CAD are atherosclerotic changes, which include vascular endothelial damage, adhesion and migration of monocytes, lipid accumulation in macrophages, and the formation of foam cells (Fredman and Spite 2013). A chronic inflammatory process is contributed to atherosclerosis. Moreover, activation of innate immunity system and chronic low-grade inflammation play important roles in all phases of atherosclerosis, including initiation, progression, and thrombotic complications (Libby 2012; Libby et al. 2010).

Toll-like receptors (TLRs) are a group of pathogen-associated molecular pattern receptors involved in innate immunity and pathogen recognition. To date, at least 13 TLRs have been discovered in mammals (O’Neill 2008). Toll-like receptor 4 (TLR4) belongs to the TLRs family and is expressed in cardiomyocytes, monocytes, endothelial cells (Zaremba and Godowski 2002) and cells of the central nervous system (Kielian 2006). TLR4 can ligate with not only the exogenous ligand lipopolysaccharide, in particular from gram-negative bacteria, but also several endogenous ligands, such as endogenous molecules released by injured tissues and necrotic cells. These molecules, called damage-associated molecular pattern molecules, induce the activation of a strong proinflammatory

*Correspondence: li_bo@jlu.edu.cn; jlyelin@163.com
¹ Department of Occupational and Environmental Health, School of Public Health, Jilin University, 1163 Xin Min Street, Changchun 130021, China
² Department of Epidemiology and Statistics, School of Public Health, Jilin University, 1163 Xin Min Street, Changchun 130021, China
Full list of author information is available at the end of the article

© 2016 The Author(s). This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.
response through interaction with TLR4 (Molteni et al. 2016). Recognition of these ligands by TLR4 contributes to activation of signaling events that can elicit the pro-inflammatory cytokine release, lipid uptake, foam cell formation and even activate adaptive immune system (Cole et al. 2010). Stimulated TLR4 signaling results in the up-regulation of TNF-α production in macrophages, what is particularly important in terms of tissue inflammation (Nagai et al. 2013). Previous studies have showed that the expression of TLR4 was markedly enhanced in human atherosclerotic plaques and this augmentation occurred preferentially on macrophages and endothelial cells (Edfeldt et al. 2002; Vink et al. 2002; Otsui et al. 2007). An animal study also validated that TLR4-deficient mice sustained significantly smaller infarctions and lower level of inflammatory responses compared with wild-type control mice (Takeishi and Kubota 2009). These revealed a fact that TLR4 might be an important susceptibility gene to CAD via its role in activation of innate immunity and inflammatory responses.

TLR4 gene is located on chromosome 9 (9q32-q33) and consists of four exons and three introns. So far, 29 single nucleotide polymorphisms (SNPs) have been identified in the TLR4 gene. With the use of SNPs as molecular markers, many genetic association studies were performed to investigate the associations of TLR4 gene polymorphisms with the risk of CAD (Kolek et al. 2004; Balisteri et al. 2004; Incalcaterra et al. 2010; Zee et al. 2005; Koch et al. 2006; Nebel et al. 2007; Džumhur et al. 2012). However, results of these studies are not always consistent. Some of them showed that TLR4 gene polymorphisms were associated with a lower risk of CAD (Kolek et al. 2004; Balisteri et al. 2004; Incalcaterra et al. 2010), while the others found no relationship between them (Zee et al. 2005; Koch et al. 2006; Nebel et al. 2007; Džumhur et al. 2012). All of above studies were performed in Caucasian populations and paid attention to two missense polymorphisms in the TLR4 gene (Asp299Gly and Thr399Ile). Because both Asp299Gly and Thr399Ile are very rare in Chinese population, there are few relevant studies about the association of TLR4 polymorphisms with CAD (Hang et al. 2004).

Considering TLR4 plays an important role in the pathogenesis of CAD, we hypothesized those common polymorphisms in the TLR4 gene might predispose human to CAD. Therefore, the aim of this study was to explore the association between tag SNPs, which capture all the essential information about the TLR4 gene locus, and CAD in a Chinese Han population.

Results

Characteristics of study subjects

The demographic and clinical characteristics of the 577 CAD patients and 517 control subjects are presented in Table 1. Compared with the control group, the CAD group had more smokers and more individuals with hypertension and with diabetes. Significant higher in age, WHR and TC was pronounced in the CAD group. There was no significant difference in gender, BMI, TG and prevalence of drinking status between the control and case groups.

TLR4 polymorphisms and CAD risk

Three SNPs (rs1927907, rs1927911 and rs11536889) in TLR4 were genotyped. All genotype distributions in both control and case groups were in Hardy–Weinberg equilibrium ($P > 0.05$, Table 2).

The allele frequencies of the 3 SNPs are listed in Table 3. There were no significant differences between healthy controls and CAD patients ($P > 0.05$). After adjustment for traditional risk factors including age, WHR, TC, history of hypertension and diabetes, and smoking status, no statistically association of the three SNPs with CAD was found between the case and control group ($P > 0.05$).

We further compared the genotypic frequencies of each SNP between the two groups by using co-dominant, dominant and recessive genetic models. After adjustment for above confounding factors, the result showed that TLR4 gene polymorphisms were not associated with the risk of CAD ($P > 0.05$, Table 3).

Haplotype analysis

Three tag SNPs selected in our study were located in one haplotype block, and the magnitude of LD between each SNP was extremely high, with pair-wise $D' > 0.94$.

Table 1 Baseline characteristics of the control and case groups

| Characteristic | Control (n = 517) | Case (n = 577) | $P$ |
|---------------|------------------|---------------|-----|
| Acute coronary syndrome, n (%) | – | 266 (46.1) | – |
| Gender, n (%) | | | |
| Female | 252 (48.7) | 270 (46.8) | 0.519 |
| Male | 265 (51.3) | 307 (53.2) | |
| Age (years)$^a$ | 63.75 ± 11.56 | 61.70 ± 12.75 | 0.080 |
| BMI (kg/m$^2$)$^b$ | 24.02 ± 3.28 | 24.11 ± 2.73 | 0.691 |
| WHR$^a$ | 0.88 ± 0.09 | 0.93 ± 0.07 | <0.001 |
| Hypertension (%) | 109 (31.6) | 245 (56.1) | <0.001 |
| Diabetes (%) | 43 (12.5) | 126 (23.8) | <0.001 |
| Smoking (%) | 84 (23.6) | 199 (37.1) | <0.001 |
| Drinking (%) | 80 (22.5) | 112 (21.2) | 0.625 |
| TC (mmol/L)$^b$ | 4.64 ± 1.22 | 5.04 ± 1.06 | <0.001 |
| TG (mmol/L)$^b$ | 1.74 ± 1.06 | 1.74 ± 1.14 | 0.611 |

$^a$ Median ± QR

$^b$ Mean ± SD
The haplotype analysis was performed to derive haplotypes specifically correlated with CAD susceptibility. As shown in Table 4, although four common TLR4 haplotypes (frequency >10 %) were found, there was no significant differences in the haplotype distributions between the control and case groups ($P > 0.05$).

Quantitative trait analysis
The levels of TC and TG in the patient group were considered as quantitative trait to analyze their relationship with the 3 SNPs, respectively. However, neither of the two plasma lipid parameters showed association for the three SNPs (Table 5).

Genotype association for hypertension and diabetes
Tables 6 and 7 analyzed the genotype association of the 3 SNPs with hypertension and diabetes in CAD patients. Neither of them showed association for the three SNPs.

Discussion
In the present study, three tag SNPs (rs1927907, rs1927911 and rs11536889) in the TLR4 gene were genotyped to investigate the association between TLR4 gene polymorphisms and the risk of CAD. The minor allele frequencies of these SNPs in the control group were 26.7, 58.6 and 20.5 %, respectively, which were similar to that in the HapMap-CHB reference population. After

---

**Table 2** Distribution of allele frequencies of SNPs in control and case groups

| SNPs      | Group   | HWE  | Allele numbers (%) | $\chi^2$ | P   | OR (95 % CI)* |
|-----------|---------|------|--------------------|---------|-----|---------------|
|           |         |      | Major allele       | Minor allele |       |               |
| rs11536889| Control | 0.454| 820 (79.5)          | 212 (20.5) | 0.506 | 0.477         | 1.104 (0.806, 1.512) |
|           | Case    | 0.619| 890 (78.2)          | 248 (21.8) |       |               |
| rs1927907 | Control | 0.926| 752 (73.3)          | 274 (26.7) | 0.583 | 0.445         | 1.095 (0.813, 1.474) |
|           | Case    | 0.542| 849 (74.7)          | 287 (25.3) |       |               |
| rs1927911 | Control | 0.713| 425 (41.4)          | 601 (58.6) | 0.161 | 0.689         | 0.910 (0.699, 1.184) |
|           | Case    | 0.272| 465 (40.6)          | 681 (59.4) |       |               |

* Adjustment for WHR, TC, history of hypertension and diabetes and smoking status

**Table 3** Association of SNPs with CAD in different genetic models

| SNPs      | Model   | Genotype | Control (%) | Case (%) | $\chi^2$ | P   | OR (95 % CI)* |
|-----------|---------|----------|-------------|----------|---------|-----|---------------|
| rs11536889| Co-dominant | G/G      | 323 (62.6)  | 346 (60.8) | 0.570  | 0.752 | 1.00         |
|           |         | G/C      | 174 (33.7)  | 198 (34.8) |        |      | 1.055 (0.714, 1.558) |
|           |         | C/C      | 19 (3.7)    | 25 (4.4)   |        |      | 1.293 (0.503, 3.326) |
|           | Dominant | G/G      | 323 (62.6)  | 346 (60.8) | 0.366  | 0.545 | 1.00         |
|           |         | G/C+C/C  | 193 (37.4)  | 223 (39.2) |        |      | 1.00 (0.755, 1.603) |
|           | Recessive| G/G+G/C  | 497 (96.3)  | 544 (95.6) | 0.352  | 0.553 | 1.00         |
|           |         | C/C      | 19 (3.7)    | 25 (4.4)   |        |      | 1.270 (0.498, 3.235) |
| rs1927907 | Co-dominant | G/G      | 276 (53.8)  | 320 (56.3) | 0.702  | 0.704 | 1.00         |
|           |         | A/G      | 200 (39.0)  | 209 (36.8) |        |      | 1.126 (0.764, 1.659) |
|           |         | A/A      | 37 (7.2)    | 39 (6.9)   |        |      | 1.139 (0.534, 2.429) |
|           | Dominant | G/G      | 276 (53.8)  | 320 (56.3) | 0.701  | 0.402 | 1.00         |
|           |         | A/G+A/A  | 237 (46.2)  | 248 (43.7) |        |      | 1.134 (0.783, 1.643) |
|           | Recessive| G/G+A/G  | 476 (92.8)  | 529 (93.1) | 0.049  | 0.824 | 1.00         |
|           |         | A/A      | 37 (7.2)    | 39 (6.9)   |        |      | 1.085 (0.517, 2.275) |
| rs1927911 | Co-dominant | T/T      | 86 (16.8)   | 88 (15.4)  | 0.409  | 0.815 | 1.00         |
|           |         | C/T      | 253 (49.5)  | 289 (50.4) |        |      | 0.844 (0.494, 1.442) |
|           |         | C/C      | 174 (33.7)  | 196 (34.2) |        |      | 0.797 (0.455, 1.398) |
|           | Dominant | T/T      | 86 (16.8)   | 88 (15.4)  | 0.398  | 0.528 | 1.00         |
|           |         | C/T+C/C  | 427 (83.2)  | 485 (84.6) |        |      | 0.824 (0.496, 1.370) |
|           | Recessive| T/T+C/T  | 340 (66.3)  | 377 (65.8) | 0.028  | 0.867 | 1.00         |
|           |         | C/C      | 173 (33.7)  | 196 (34.2) |        |      | 0.926 (0.630, 1.361) |

* Adjustment for WHR, TC, history of hypertension and diabetes and smoking status
systemic analysis, we found no evidence to support a significant association between TLR4 gene polymorphisms and CAD susceptibility.

TLR4 is a type 1 transmembrane protein that mediates immune responses to both endogenous and exogenous ligands (O’Neill 2008). On binding of the specific ligands, TLR4 triggers signal transduction that induces the production and secretion of pro-inflammatory cytokines and chemokines through myeloid differentiation primary-response protein 88 (MyD88) or TIR domain-containing adaptor inducing IFN-β (TRIF) dependent signaling pathways, as appropriate (Seneviratne et al. 2012). Therefore, TLR4 plays a crucial role in the pathogenesis of CAD, while genetic variations within the gene have an important influence on the pathogenesis. A polymorphism Asp299Gly, resulting in amino acid exchange in the extracellular domain of the receptor, is associated with a blunted inflammatory response (Arbour et al. 2000). This might lead to altered risk of CAD. Previous studies have been performed to investigate the association of the polymorphism Asp299Gly with CAD susceptibility in Caucasian populations, albeit with conflicting results. Some of these studies showed that the minor G allele was associated with a reduced risk of CAD and acute coronary events (Kolek et al. 2004; Balistreri et al. 2004; Incalcaterra et al. 2010; Ameziane et al. 2003; Edfeldt et al. 2004; Kutikhin et al. 2016; Boekholdt et al. 2003; Holloway and Yang 2005), while the others found no relationship between them (Zee et al. 2005; Koch et al. 2006; Nebel et al. 2007; Džumhur et al. 2012; Golovkin et al. 2014; Morange et al. 2004; Hernesniemi et al. 2006; Lima-Neto et al. 2013). A meta-analysis indicated that Asp299Gly polymorphism was not associated with MI risk (Yin et al. 2014). However, the prevalence of Asp299Gly polymorphism is very low in Chinese population. Liu et al. (2012) and Lin et al. (2005) even failed to detect the presence of any variant for the SNP. So we did not investigate the role of the less common non-synonymous SNP in our study. Beside Asp299Gly polymorphism within TLR4 gene, a study investigated the association between four new substitutions found by re-sequencing in the 5 V-proximal promoter region of the TLR4 gene and acute myocardial infarction, but they did not find the association (De Staercke et al. 2007). We selected three tag SNPs for genetic analysis, including rs1927907, rs1927911 and rs11536889. Although any of them are not in exon, they are useful to find the disease-related variants because they capture abundant genetic information in the TLR4 gene based on the CHB dbSNP database.

Two SNPs, rs1927907 and rs1927911, are located in intron. Introns are identified to have evolved to function as endogenous network control molecules, enabling direct gene–gene communication and multitasking of eukaryotic genomes, and are important in genetic expression and regulation (Mattick and Gagen 2001). A report regarding the relationship between the TLR4 gene polymorphisms and late-onset Alzheimer’s disease (LOAD) showed that participants with AA genotype of rs1927907 had a significantly increased risk of LOAD (Chen et al. 2012). The minor allele C of rs1927911 was previously demonstrated to be associated with normal tension glaucoma (Takano et al. 2012; Shibuya et al. 2008), cancer (Zhang et al. 2013; Song et al. 2009) and pulmonary tuberculosis (Zaki et al. 2012). In our study, however, no statistically association was found between genetic variations in the rs1927907 and rs1927911 locus and the risk of CAD. The result is inconsistent with a relevant study performed in a Washington population, which revealed that minor allele C of rs1927911 was associated with a

| Lipid | SNPs       | Major allele (%) | Minor allele (%) | Variance | χ²     | P   |
|-------|------------|------------------|------------------|----------|--------|-----|
| TC    | rs11536889 | 890 (78.2)       | 248 (21.8)       | 2.002    | 2.448  | 0.118 |
|       | rs1927907 | 849 (74.7)       | 287 (25.3)       | 2.076    | 1.306  | 0.253 |
|       | rs1927911 | 464 (40.5)       | 682 (59.5)       | 2.050    | 0.005  | 0.944 |
| TG    | rs11536889 | 890 (78.2)       | 248 (21.8)       | 1.321    | 3.072  | 0.788 |
|       | rs1927907 | 849 (74.7)       | 287 (25.3)       | 1.331    | 0.309  | 0.578 |
|       | rs1927911 | 464 (40.5)       | 682 (59.5)       | 1.311    | 0.688  | 0.407 |

Table 4 Haplotype frequencies of SNPs in control and case groups

| Haplotype a | Control (%) | Case (%) | χ² | P   |
|-------------|-------------|----------|-----|-----|
| rs1927911-rs1927907-rs11536889 |             |          |     |     |
| CGC         | 206 (20.2)  | 236 (21.2) | 0.420 | 0.517 |
| CGG         | 392 (38.5)  | 427 (38.3) | 0.014 | 0.907 |
| TAG         | 271 (26.6)  | 279 (25.0) | 0.575 | 0.449 |
| TGG         | 147 (14.4)  | 166 (14.9) | 0.088 | 0.767 |

Global: χ² = 3.557, df = 6, P = 0.736

a Haplotypes with frequency >10% were listed

Table 5 Quantitative trait analysis for allelic association of the 3 SNPs with plasma lipid levels in patients with CAD
12% lower MI risk (Enquobahrie et al. 2008). The reason which can account for this contradictory result is the ethnic difference. The previous study was performed in a Caucasian population, while our study in a Chinese Han population.

The SNP rs1156889 is located in the 3′-UTR of the TLR4 gene, where the sequence may influence mRNA stability, translation and localization and thereby regulate expression of the gene and interfere with the host immune system. In previous studies, the rs11536889 C was showed to be significantly associated with a high risk of emphysema (Ito et al. 2012) and prostate cancer (Kim et al. 2012). However, our study did not find any significant association between this SNP and CAD, relating to allele, genotype and haplotype analysis.

As a complex disease, there are overly confounding variables and genetic mechanisms that can lead to CAD. Analyzing the genetic variants can probably represent the first step to understand the pathophysiology of CAD, but can not explain the whole effect of TLR4 on CAD. Conditions of patients with CAD, such as hypertension, diabetes and plasma lipid levels, may influence the effect. Schneider S et al. had demonstrated an association between the TLR4 SNP rs4986790 genotype and age-dependant blood pressure increase in patients with coronary artery disease (Schneider et al. 2015). In order to take into consideration these factors affecting TLR4 genetic analysis in CAD, We investigate the combined effect of TLR4 and some common risk factors on the development of the disease. The results showed that there was no genotype association for plasma lipid levels, hypertension and diabetes. This suggested the plasma lipid levels, hypertension and diabetes did not influence the effect of TLR4 on CAD.

### Table 6 Genotype association of the 3 SNPs with hypertension in patients with CAD

| SNPs     | Major-major | Major-minor | Minor-minor | χ² | P  |
|----------|-------------|-------------|-------------|----|----|
|          | No (%)      | Yes (%)     | No (%)      | Yes (%) | No (%) | Yes (%) |    |    |
| rs11536889 | 45.9        | 54.1        | 33.8        | 68.2 | 44.0    | 56.0    | 2.072 | 0.355 |
| rs1927907  | 40.7        | 59.3        | 48.1        | 51.9 | 42.9    | 57.1    | 2.202 | 0.333 |
| rs1927911  | 42.0        | 48.0        | 43.9        | 46.1 | 46.8    | 43.2    | 0.417 | 0.812 |

### Table 7 Genotype association of the 3 SNPs with diabetes mellitus in patients with CAD

| SNPs     | Major-major | Major-minor | Minor-minor | χ² | P  |
|----------|-------------|-------------|-------------|----|----|
|          | No (%)      | Yes (%)     | No (%)      | Yes (%) | No (%) | Yes (%) |    |    |
| rs11536889 | 78.4        | 21.6        | 71.0        | 29.0 | 75.0    | 25.0    | 3.448 | 0.178 |
| rs1927907  | 74.8        | 25.2        | 76.7        | 23.3 | 85.7    | 14.3    | 2.084 | 0.353 |
| rs1927911  | 76.8        | 23.2        | 74.0        | 26.0 | 81.2    | 18.8    | 1.871 | 0.392 |

Conclusion
The present study suggests that TLR4 polymorphisms are not associated with CAD in the Chinese Han population. This study, to our knowledge, is the first attempt to assess the association of the three tag SNPs in TLR4 gene with CAD in Chinese Han population and must contribute valuable information to the future relevant studies.

Methods
Study population
A total of 577 unrelated patients with CAD were recruited from the First Hospital of Jilin University between 2008 and 2012, including 266 patients with acute coronary syndrome. CAD was defined as the result of standardized coronary angiography and the diagnostic criteria of it was a ≥50% stenosis in one or more main coronary arteries. All the angiographies were interpreted with the consensus from at least two independent cardiologists who were blinded to the results of the genetic analysis. Individuals were excluded if they suffered from congenital heart disease, cardiomyopathy, hepatic or renal disease and cancer. Control subjects (n = 517), residing in the same geographical area as the cases, were randomly selected from the persons who were through the routine checkup as part of annual body examination. They were classified as healthy controls based on the results of physical examination coupled with the absence of any reasons to suspect CAD.

Clinical data, including age, gender, body mass index (BMI), waist-to-hip ratio (WHR), hypertension, diabetes mellitus, smoking and drinking status, total cholesterol (TC) and triglyceride (TG), were obtained from all participants. Blood samples were collected in tubes containing EDTA and stored at −80°C until extraction.
Every subject gave a written informed consent and was well told of the study protocol. The study was approved by the ethics committee of school of public health, Jilin University, Changchun, China.

**SNPs selection and genotyping**

The data of reported TLR4 SNPs for Chinese Han population in Beijing was obtained from the International HapMap Project (www.hapmap.org). Based on the criteria of minor allele frequencies (MAFs) ≥0.10 and linkage disequilibrium of $r^2 > 0.8$, three SNPs were selected for genetic analysis. There are rs1927907 and rs1927911, located in the intron of TLR4 gene, while rs11536889 in the 3′ untranslated region.

Genomic DNA was extracted from peripheral blood lymphocytes using a DNA extraction kit (TianGen, Beijing, China). Genotypes of SNPs were detected using Sequenom Mass-ARRAY system with genotyping success rate greater than 96% for each SNP. The amplification primers and extension primers are listed in Table 8. For quality control, 30 random samples were genotyped repetitively together with others, and the concordance rate was 100%.

Continuous variables were expressed as median ± QR or mean ± SD and were compared by student’s t test or the Mann–Whitney U test, as appropriate. Categorical variables were expressed as counts (percentage) and were compared with Pearson $\chi^2$ test. The Hardy–Weinberg equilibrium for the genotypic distributions of SNPs was tested using the $\chi^2$ goodness-of-fit test. The unconditional logistic regression models were performed to calculate the odds ratios (OR) and 95% confidence intervals (CI). Three inheritance models (co-dominant, dominant and recessive) were defined and were applied to genotypic analysis. Genotype association for diabetes mellitus and hypertension was performed with $\chi^2$ test. The values of linkage disequilibrium (LD) between all pairs of biallelic loci were calculated using Haploview software (Version 4.2). Haplotypic analysis and quantitative trait tests were performed with the UNPHASED program (Version 3.0.12). A two-tailed $P$ value <0.05 was considered to be statistically significant. Statistical analysis was carried out using SPSS 13.0 for Windows software.

### Table 8 Amplification and extension primers used for genotyping TLR4 gene

| SNPs     | Forward primers       | Reverse primers       | Extension primers     |
|----------|-----------------------|-----------------------|-----------------------|
| rs11536889 | ACGTTGAGTATTTCTGTGGGCAATGCTC | ACGTTGAGTACCCCTAACATTCCAGACAC | TTTTTCTCAATGATAAACATCCACTC |
| rs1927907  | ACGTTGAGTATAGGGACTACCCCTCC | ACGTTGAGTCCGGATTTAGCAGGAAGA | GGAATTACATAAAGACATGGTTGA |
| rs1927911  | ACGTTGAGTATCTATGCTCAAGGTTC | ACGTTGAGTCCACCTCTGCTCAGGG | AGAGTTGACACGTCCTTTTT |

**Abbreviations**

BMI: body mass index; CAD: coronary artery disease; CI: confidence intervals; LD: linkage disequilibrium; LOAD: late-onset Alzheimer’s Disease; MAFs: minor allele frequencies; OR: odds ratios; SNPs: single nucleotide polymorphisms; TC: total cholesterol; TG: triglyceride; TLR4: Toll-like receptor 4; TLRs: Toll-like receptors (TLRs); WHR: waist-to-hip ratio.

**Authors’ contributions**

LY designed the study that led to this article. LZ recruited the subjects, selected and genotyped the SNPs. DZ contributed to data collection, manuscript preparation and DNA extraction. SW and JZ participated in blood samples collected. BL analyzed the relationship between genotype of the 3 SNPs and the lipid level, hypertension and diabetes. Other authors listed all participated in DNA extraction. All authors read and approved the final manuscript.

**Acknowledgements**

This work was sponsored by the Specialized Research Fund for the Doctoral Program of Higher Education of China (20100061110071). We thank the participants for their participation and also thank the First Hospital of Jilin University for providing blood samples and detailed information of participants.

**Competing interests**

The authors declare that they have no competing interests.

**References**

Ameziane N, Beillat T, Verpillat P et al (2003) Association of the Toll-like receptor 4 gene Asp299Gly polymorphism with acute coronary events. Atheroscler Thromb Vasc Biol 12:e61–e64

Arbour NC, Lorenz E, Schutte BC et al (2008) TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 25:187–191

Balistieri CR, Candore G, Colonna-Romano G et al (2004) Role of Toll-like receptor 4 in acute myocardial infarction and longevity. JAMA 292:2339–2340

Boekholdt SM, Agema WR, Peters RJ et al (2003) Variants of toll-like receptor 4 modify the efficacy of statin therapy and the risk of cardiovascular events. Circulation 119:2416–2421

Chen YC, Yip PK, Huang YL et al (2012) Sequence variants of toll-like receptor 4 and late-onset Alzheimer’s disease. PLoS ONE 7:e50771

Cole JE, Georgiou E, Monaco C (2010) The expression and functions of toll-like receptors in atherosclerosis. Mediat Inflamm 2010:393946

De Staercke C, Lally C, Austin H, Winston C et al (2007) The lack of association between four point mutations in the promoter region of the toll-like 4 receptor gene and myocardial infarction. Thromb Res 113:105–110
Džumurh A, Zibar L, Wagner J et al (2012) Association studies of gene polymorphisms in toll-like receptor 2 and 4 in Croatian patients with acute myocardial infarction. Scand J Immunol 75:517–523

Edfeldt K, Swedenborg J, Hansson GK et al (2002) Expression of toll-like receptors in human atherosclerotic lesion: a possible pathway for plaque activation. Circulation 105:1158–1161

Edfeldt K, Bennet AM, Eriksson P et al (2004) Association of hypo-responsive toll-like receptor 4 variants with risk of myocardial infarction. Eur Heart J 16:1447–1453

Enoubabahri DA, Smith NL, Bis JC et al (2008) Cholesterol ester transfer protein, interleukin-8, peroxisome proliferator activator receptor alpha, and Toll-like receptor 4 genetic variations and risk of incident nonfatal myocardial infarction and ischemic stroke. Ann J Cardiol 101:1683–1688

Fredman G, Spite M (2013) Recent advances in the role of immunity in atherosclerosis. Circ Res 12:23–114

Golovkin AS, Ponasenko AV, Khutornaya MV et al (2014) Association of TLR and TREM-1 gene polymorphisms with risk of coronary artery disease in a Russian population. Gene 1:101–109

Hang J, Zhou W, Zhang H et al (2004) TLR4 Asp299Gly and Thr399Ile polymorphisms are very rare in the Chinese population. J Endotoxin Res 10:238–240

Hernesniemi J, Lehmitmäki T, Ronu R et al (2006) Toll-like receptor 4 polymorphism is associated with coronary stenosis but not with the occurrence of acute coronary myocardial infarctions. Scand J Clin Lab Invest 8:667–675

Holloway JW, Yang IA, Ye S (2005) Variation in the toll-like receptor 4 gene and susceptibility to myocardial infarction. Pharmacogenet Genomics 11:15–21

Incalcaterra E, Caruso M, Balistreri CR et al (2010) Role of genetic polymorphisms in myocardial infarction at young age. Clin Hemorheol Microcirc 46:291–298

Ito M, Hanaoka M, Droma Y et al (2012) The association of Toll-like receptor 4 gene polymorphisms with the development of emphysema in Japanese subjects: a case control study. BMC Res Notes 5:36

Kielland T (2006) Toll-like receptors in central nervous system glial inflammation and homeostasis. J Neurosci Res 5:711–730

Kim HJ, Bae JS, Chang HI et al (2012) Sequence variants of Toll-like receptor 4 (TLR4) and the risk of prostate cancer in Korean men. World J Urol 30:225–232

Koch W, Hoppmann P, Pfeuffer A et al (2006) Toll-like receptor 4 gene polymorphisms and myocardial infarction: no association in a Caucasian population. Eur Heart J 27:2524–2529

Kolek MJ, Carlquist JF, Muhlestein JB et al (2004) Toll-like receptor 4 gene Asp299Gly polymorphism is associated with reductions in vascular inflammation, angiographic coronary artery disease, and clinical diabetes. Am Heart J 148:1034–1040

Kutikhin AG, Ponasenko AV, Khutornaya MV et al (2016) Association of TLR and TREM-1 gene polymorphisms with atherosclerosis severity in a Russian population. Meta Gene 9:76–89

Libby P (2012) Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol 32:2045–2051

Libby P, Okamoto Y, Dobrzynski H et al (2010) Inflammation in atherosclerosis: transition from theory to practice. Circ J 74:213–220

Lima-Neto LG, Hirata RD, Luchessi AD et al (2013) CD14 and IL6 polymorphisms are associated with a pro-atherogenic profile in young adults with acute myocardial infarction. J Thromb Thrombolysis 35:332–340

Lin YC, Chang YM, Yu JM et al (2005) Toll-like receptor 4 gene C119A but not Asp299Gly polymorphism is associated with ischemic stroke among ethnic Chinese in Taiwan. Atherosclerosis 180:305–309

Liu F, Lu W, Qian Q et al (2012) Frequency of TLR2, 4, and 9 gene polymorphisms in Chinese population and their susceptibility to type2 diabetes and coronary artery disease. J Biomed Biotechnol 2012:373945

Mattick JS, Gajgen MJ (2001) The evolution of controlled multitranscribed gene networks: the role of introns and other noncoding RNAs in the development of complex organisms. Mol Biol Evol 18:1611–1630

Molteni M, Gemma S, Rossetti C (2016) The role of Toll-Like Receptor 4 in infectious and noninfectious inflammation. Mediat Inflamm 2016:6978936

Morange PE, Tietz L, Saut N et al (2004) TLR4/Asp299Gly, CD14/C-260T, plasma levels of the soluble receptor CD14 and the risk of coronary heart disease: the PRIME study. Eur J Hum Genet 12:1041–1049

Nagai Y, Watanabe Y, Takatsu K (2013) The TLR family protein RP105/M0-1 complex: a new player in obesity and adipose tissue inflammation. Adipocyte 2:61–66

Nebel A, Flachsbart F, Schäfer A et al (2007) Role of the toll-like receptor 4 polymorphism Asp299Gly in longevity and myocardial infarction in German men. Mech Ageing Dev 128:409–411

O’Neill LA (2008) The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 226:10–18

Otsu K, Inoue N, Kobaaya S et al (2007) Enhanced expression of TLR4 in smooth muscle cells in human atherosclerotic coronary artery. Heart Vessels 22:416–422

Roger VL, Go AS, Lloyd-Jones DM et al (2012) Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation 125:e2–e220

Schneider S, Koch W, Hoppmann P et al (2015) Association of Toll-like receptor 4 polymorphism with age-dependent systolic blood pressure increase in patients with coronary artery disease. Immun Ageing 12:4

Seneviratne AN, Sivagurunathan B, Monaco C (2012) Toll-like receptors and macrophage activation in atherosclerosis. Clin Chim Acta 413:3–14

Shibuya E, Iseguro A, Ota M et al (2008) Association of Toll-like receptor 4 gene polymorphisms with normal tension glaucoma. Invest Ophthalmol Vis Sci 49:4453–4457

Song J, Kim DY, Kim CS et al (2009) The association between Toll-like receptor 4 (TLR4) polymorphisms and the risk of prostate cancer in Korean men. Cancer Genet Cyto genet 190:88–92

Takano Y, Shi D, Shimizu A et al (2012) Association of Toll-like receptor 4 gene polymorphisms in Japanese subjects with primary open-angle, normal-tension, and exfoliation glaucoma. Am J Ophthalmol 154:825–832

Takishita Y, Kubota I (2009) Role of Toll-like receptor mediated signaling pathway in ischemic heart. Front Biosci 14:2553–2558

Vink A, Schoneveld AH, van der Meer JJ et al (2002) In vivo evidence for a role of toll-like receptor 4 in the development of intimal lesions. Circulation 106:1985–1990

Yin YY, Sun QQ, Hu AM et al (2014) Toll-like receptor 4 gene Asp299Gly polymorphism in myocardial infarction: a meta-analysis of 15,148 subjects. Hum Immunol 2:163–169

Zaki HY, Leung KH, Yiu WC et al (2012) Common polymorphisms in TLR4 gene polymorphisms in Japanese subjects with primary open-angle, normal-tension, and exfoliation glaucoma. Am J Ophthalmol 154:825–832

Zakai HY, Leung KH, Yu WC et al (2012) Common polymorphisms in TLR4 gene polymorphisms in Japanese subjects with primary open-angle, normal-tension, and exfoliation glaucoma. Am J Ophthalmol 154:825–832

Zaremba KA, Godowski PJ (2002) Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol 168:554–561

Zee RT, Hegener HH, Gould J et al (2005) Toll-like receptor 4 Asp299Gly gene polymorphism and risk of atherothrombosis. Stroke 36:154–157

Zhang K, Zhou B, Wang Y et al (2013) The TLR4 gene polymorphisms and susceptibility to cancer: a systematic review and meta-analysis. Eur J Cancer 49:946–954

Submit your manuscript to a SpringerOpen journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Immediate publication on acceptance
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article