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# The Correlation of Increased CRP Levels with NFKB1 and TLR2 Polymorphisms in the Case of Morbid Obesity

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## Abstract

Morbid obesity (MO) is associated with an increase in circulating levels of systemic acute phase proteins such as C-reactive protein (CRP). Toll-like receptor is possible candidate for inflammatory responses which is mainly mediated by NFKB1. The aim of this study was to investigate the relationship between NFKB1 and Toll-like receptor (TLR) 2 polymorphisms and the risk of MO in a Turkish population in the context of CRP serum levels which may contribute to susceptibility to the disease. We analysed the distribution of NFKB1-94 ins/del ATTG rs28362491 and TLR2 Arg753Gln rs5743708 polymorphisms using PCR-RFLP method and CRP serum levels using ELISA method in 213 MO and 200 healthy controls. The frequency of the ins/ins genotype and ins allele of rs28362491 was significantly higher in the patients compared to control group ($P$: 0.0309; $P$: 0.0421, respectively). Additionally, the frequency of GG genotype and G allele of rs5743708 was found to be statistically higher in the patient group ($P$: 0.0421; $P < 0.0001$, respectively). In addition, serum CRP levels (>20 mg/l) in MO patients with ins/ins genotype were significantly higher than in patients with del/ins genotype ($P$: 0.0309). Serum CRP levels were also higher in MO patients with GG genotype and G allele ($P$: 0.0001). According to combined analysis, the wild type of rs28362491 and rs5743708 polymorphisms (ins/ins/GG genotype) was also significantly higher in the patient group versus the control group when compared with the combined ins/ins/GA and del/ins/GA genotype ($P < 0.0001$). Therefore, our findings suggest that rs28362491 and rs5743708 polymorphisms were significantly associated with MO disease through acting by modulating serum CRP levels.

## Introduction

Obesity is a metabolism disorder associated with low-grade chronic inflammation, characterized by an increase in circulating levels of systemic acute phase proteins such as C-reactive protein (CRP), cytokines and other interleukins [1, 2]. Morbid obesity (MO) in humans [body mass index (BMI) (the weight in kilograms divided by the square of the height in metres)] instead appears to have a particularly strong genetic component. Recently, there has been rising interest in the role of inflammation in the morbid obese. CRP, an acute phase reactant, has undergone intense investigation. CRP is a marker frequently measured in clinical practice to evaluate systemic inflammation especially in situations of infection [3]. Studies have reported positive correlations between CRP and BMI [4]. In clinical practice, with obese patients presenting with high CRP levels above 15–20 mg/l, extensive clinical investigations are frequently undertaken to identify the underlying cause of elevated CRP. This seems particularly the case in patients with MO for which the determinants of high CRP concentrations have not been thoroughly studied [2].

Many genes encoding pro-inflammatory and some genes encoding anti-inflammatory proteins in adipose tissues are regulated by the NFKB transcription factor family [5, 6]. The NFKB family consists of heterodimeric or homodimeric combinations of five subunits (p50, p100, RelA (p65), RelB and c-Rel), and different combinations have different target genes. The p50/p50 homodimer represses transcription of pro-inflammatory cytokines like CRP [6, 7]. Many researchers have pointed that variations within the *NFKB1* gene could potentially influence the function of NFKB protein and in turn the process of inflammation. Not surprisingly, *NFKB1*-94 ins/del ATTG promoter polymorphism which encodes p50 subunit was investigated in many metabolic and inflammatory diseases such as





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Behcet's disease [8], Graves' disease [9], Hashimoto thyroiditis [10], type 1 diabetes mellitus [11] and obesity [12].

Toll-like receptor (TLR) 2 at the surface of innate immune cells leads to the activation of inflammatory pathways mediated by the proinflammatory transcription factor, NFKB, and the secretion of proinflammatory cytokines and their mediators [13, 14]. Toll-like receptors are pattern recognition receptors that were originally characterized in the innate immune system. TLRs are activated by pathogen-associated molecular patterns, such as bacterial cell wall components, and induce inflammatory responses [15]. The potential biological and clinical significance of TLR2 receptor has been reported in several inflammatory disorders such as cardiovascular disease [16, 17] and children obesity [18]. Obesity leads to the enhanced expression of *TLR2* on the peripheral blood mononuclear cells, which has significant implications with regard to inflammation [19].

Certain single nucleotide polymorphisms (SNPs) in the TLR genes have been demonstrated to impair the inflammatory and immune responses to their respective ligands [20, 21]. Investigating TLR polymorphisms is of interest because they may lead to decreased expression of downstream inflammatory cytokines [18]. The common SNP in the *TLR2* gene is a substitution of arginine for glutamine at position 753 (Arg753Gln) that results in defective intracellular signalling and impaired cytokine secretion in response to peptidoglycan, lipopeptides and other known ligands [22, 23].

In the light of these data, polymorphisms in *NFKB1* and *TLR2* gene would be attractive candidates for searching MO risk, opening the question whether individual variations in the expression of these genes might account for different susceptibilities to MO in the context of CRP levels. Therefore, we analysed the association of SNPs in *NFKB1* and *TLR2* genes with susceptibility to MO in a group of women in Turkish population as a case–control study. Secondly, we investigated CRP levels according to the genotypes of both *NFKB1*-94 ins/del ATTG and *TLR2* Arg753Gln polymorphisms as single and combined in patient group.

## Patients and methods

*Subjects*. The study enrolled 213 female morbid obese patients and 200 female healthy subjects at the Department of Endocrinology and Metabolism, Bezmialem University Hospital, Istanbul, Turkey [Project No: 6.2015/7 (2015)]. The mean age was 38.0 ± 5.7 years in patients and 39.0 ± 7.3 in control group. The obesity was diagnosed according to BMI. The patients with BMI > 35 kg/m$^2$ and the healthy subjects with BMI < 25 kg/m$^2$ were included to the study. The healthy subjects having any systemic disease including chronic kidney, liver or cardiovascular diseases, or malignancies, rheumatologic disorders or any inflammatory diseases were excluded from the study. All study subjects have Turkish origin and provided signed informed consent prior to the sample and data collection. Study protocol was approved by Institutional Ethical Committee of Bezmialem University with decision number of 03/06/2015 - 11/10.

*Blood samples and DNA isolation*. Venous blood samples were collected in plastic vacutainer tubes, either containing EDTA for DNA isolation and without additives for serum CRP level. Immediately after collection, serum CRP levels are measured. Whole blood samples were stored at −20 °C for genomic DNA extraction. Genomic DNA was extracted from blood using Roche DNA purification kit (Product No. 11796828001; Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The concentration and purity of DNA samples were established using a Nanophotometer Pearl (Implen, Inc. Westlake Village, CA 91362, USA). DNA samples with OD ratio 1.8 ± 0.1 were included in the study.

*Polymorphism analysis*. The polymorphisms of *NFKB1* gene were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. 285/281-base pair PCR fragment of the *NFKB1* gene was amplified in a 25 μl reaction volume containing 100 ng genomic DNA, 200 pmol of each dNTPs, 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1 U Taq Polymerase (Sigma, St. Louis, MO, USA) and 2 mM $MgCl_2$ (Fermantas, Vilnius, Lithuania). Primers were F: 5′-TGGGCACAA GTCGTTTATGA-3′ and R: 5′-CTGGAGCCGGTAGGG AAG-3′. PCR conditions were as follows: 95 °C for 1 min; 35 cycles of 95 °C for 30 s, 61 °C for 30 s, 72 °C for 1 min; and final incubation at 72 °C for 5 min. The products were digested overnight with 5 U of *PflMI* (*Van91I*) (Fermentas) at 37 °C and were run on an ethidium bromide-stained 3% agarose gel for 45 min at 90 V and were directly screened under UV light to detect two different alleles of rs28362491, the 281 bp (deletion allele) or 285 bp (insertion allele). As deletion genotype has no *PflMI* (*Van91I*) restriction site, the PCR product of 281 bp remained undigested. On the other hand, the insertion variants were cleaved by PflMI (Van91I) restriction enzyme into two fragments of 240 bp and 45 bp. Heterozygotes showed all three bands (Fig. 1).

Genotyping for the *TLR2* Arg753Gln polymorphism was also carried out by PCR-RFLP method. Genomic DNA was amplified with a forward primer of 5′-CAGCGCTTCTGCAAGCTCC-3′ and a reverse primer of 5′-CTTTATCGCAGCTCTCAGATTTACCC-3 to have a 111-bp product. PCR conditions were as follows: 95 °C for 2 min; 35 cycles of 94 °C for 30 s, 61 °C for 45 s, 72 °C for 45 s; and final incubation at 72 °C for 3 min. For RFLP, the products were digested with 5 U of *MspI* (New England Biolabs, Ipswich, MA) at 37 °C for 15 min and were run on an ethidium bromide-stained 3% agarose gel

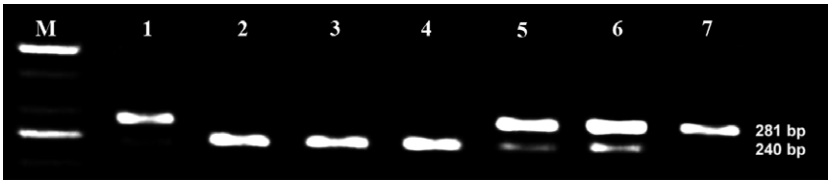


**Figure 1** The enzyme digestion pattern of rs28362491. M is 50-bp size marker; lanes 5 and 6 are heterozygous ins/del ATTG; lanes 2, 3 and 4 are homozygous ins/ins ATTG; lanes 1 and 7 are del/del ATTG homozygous.

for 45 min at 90 V and were directly screened under UV light. The heterozygous genotype (GA) gives three bands of 111 bp, 93 bp and 18 bp, as only one strand of amplicon is cut. The homozygous mutant genotype (AA) is observed as a single band with 111 base pair, whereas homozygote wild-type genotype (GG) is observed as two bands of 93 and 18 base pairs (Fig. 2).

*Determination of CRP levels*. In serum samples of patients, hs-CRP (DIAsource, Louvain-la-Neuve, Belgium) test was run under the control of clinicians in accordance with the instructions of the manufacturers.

*Statistical analysis*. GRAPHPAD PRISM 5 (GraphPad Software, Inc. CA, USA) program was used for the analysis of the patients and control values. Hardy–Weinberg equilibrium (HWE) was tested by chi-square analysis. Genotype and allele frequencies were compared between the cases and the controls by chi-square analysis. Odds ratio (OR) and respective 95% confidence intervals (CIs) were reported to evaluate the effects of any difference between allelic and genotype distribution. A two-sided $P$ value $\leq 0.05$ was considered statistically significant. The confidence in the results will generally be weaker if it is conducted as part of a multiple comparison analysis, rather than a single comparison analysis.

## Results

### Association of the SNPs and morbid obesity risk

Table 1 showed the association of the SNPs and MO risk. The distribution of *NFKB1* rs28362491 but not *TLR2* rs5743708 genotypes in the control group was within the HWE (Table 2). We found a significant association between rs28362491 and rs5743708, and MO. The frequency of the ins/ins genotype of rs28362491 was significantly higher in the patients compared to control group (OR: 1.583, 95% CI: 1.042–2.404, $P = 0.0309$). According to our results, having ins/ins genotype of rs28362491 has 1.583 times risk factor in patients with MO. When the allele frequencies were compared, we found that having the ins allele increased the risk for MO by 1.350-fold (OR: 1.350, 95% CI: 1.010–1.803, $P = 0.0421$).

For *TLR2* rs5743708 polymorphism, the GG genotype was associated with a higher MO risk, with adjusted OR:

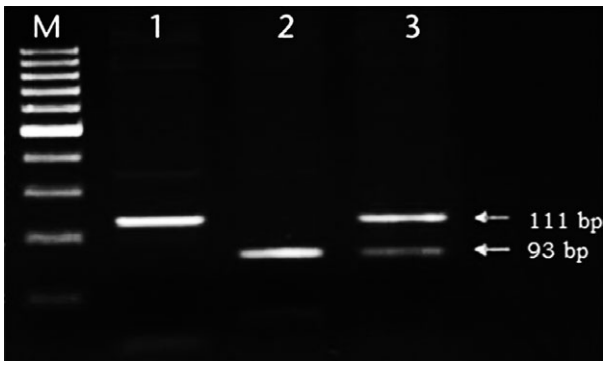


**Figure 2** The enzyme digestion pattern of rs5743708. M is 50-bp size marker; Lane 1 is mutant type homozygous alleles (A/A); Lane 2 is wild-type homozygous alleles (G/G); and Lane 3 is heterozygous alleles (G/A).

**Table 1** The distribution of genotype and allele frequencies of rs28362491 and rs5743708 polymorphisms in morbid obesity patients and control subjects.

| | Patient *n* (%) | Control *n* (%) | OR (%95 CI) | *P* |
|---|---|---|---|---|
| NFKB | | | | |
| ins/ins | 92 (46.5) | 73 (35) | 1 | |
| del/ins | 86 (43.5) | 108 (52) | 1.583 (1.042–2.404) | **0.0309** |
| del/del | 20 (10) | 26 (13) | 1.638 (0.8475–3.167) | 0.1906 |
| ins allele | 270 (68) | 254 (61) | 1 | |
| del allele | 126 (32) | 160 (39) | 1.350 (1.010–1.803) | **0.0421** |
| TLR2 | | | | |
| GG | 213 (100) | 144 (74) | 1 | |
| GA | 0 (0) | 39 (20) | 116.7 (7.112–1916) | **0.0001** |
| AA | 0 (0) | 11 (6) | 33.98 (1.985–581.6) | **0.0003** |
| G allele | 426 (100) | 327 (84) | 1 | |
| A allele | 0 (0) | 61 (16) | 160.2 (9.864–2601) | **0.0001** |

Statistically significant values ($p < 0.05$) are marked in bold.

**Table 2** Allele frequencies and HWE *P* values.

| | Patient | Control | Total |
|---|---|---|---|
| NFKB1 | | | |
| ins/ins | 92 (46.5) | 73 (35) | 165 (41) |
| del/ins | 86 (43.5) | 108 (52) | 194 (48) |
| del/del | 20 (10) | 26 (13) | 46 (11) |
| HWE *P* value | 0.9881 | 0.1494 | 0.3285 |
| TLR2 | | | |
| GG | 213 (100) | 144 (74) | 357 (88) |
| GA | 0 (0) | 39 (20) | 39 (9.5) |
| AA | 0 (0) | 11 (6) | 11 (2.5) |
| HWE *P* value | 0.3285 | 0.0008 | 0 |

HWE, Hardy–Weinberg Equilibrium.

116.7, 95% CI: 7.112–1916, $P < 0.0001$ for GA and adjusted OR: 33.98, 95% CI: 1.985–581.6, $P = 0.0003$ for AA. Additionally, the frequency of G allele of rs5743708 was found to be statistically higher in the patient group (OR: 160.2, 95% CI: 9.864–2601, $P < 0.0001$).

Table 3 represents the distribution of the allele and genotype frequencies of the *NFKB1* and *TLR2* polymorphisms in control group and MO patients that divided into two groups: LI (CRP < 20 mg/l) and HI (CRP ≥ 20 mg/l) groups. According to our results, having ins/ins genotype of rs28362491 has 1.82 times risk for heterozygotes in HI with MO group (OR: 1.828, 95% CI: 1.064–3.140, $P = 0.0280$), whereas there were no significant differences in the distribution of rs28362491 genotype and allele frequencies between LI with patients and control group ($P > 0.05$).

Furthermore, when we compared serum CRP levels according to genotypes of TLR2 rs5743708 polymorphism in two patients groups (HI and LI), the frequency of GG genotype and G allele was significantly higher in both patient groups compared to controls ($P < 0.0001$; $P < 0.0001$, respectively).

GG genotype was associated with a higher risk when compared with GA and AA genotypes ($P < 0.0001$, OR = 47.29; $P < 0.0273$, OR = 13.77; respectively) for LI and ($P < 0.0001$, OR = 40.18; $P < 0.0453$, OR = 11.70; respectively) for HI. In addition, the frequency of G allele of rs5743708 was found to be a higher risk, with OR: 64.79, 95% CI: 3.980–1055, $P < 0.0001$ in LI MO group and with OR: 55.02, 95% CI: 3.378–896.3, $P < 0.0001$ in HI MO group.

### Combined effect of NFKB1 and TLR2 polymorphisms

NFKB1 and TLR2 polymorphisms were screened and genotyped and their association with the risk of MO was assessed using logistic regression analysis.

Table 4 summarizes the association studies among the combined genotypes of the two SNPs and the overall risk for MO. The *NFKB1* rs28362491 and *TLR2* rs5743708 SNPs showed statistical significance among the combined genotypes in MO patients and controls. The combined del/ins/GG genotype and del/del/GG frequencies were significantly higher in the control group versus the patient group when compared with the combined ins/ins/GG, ins/ins/GA, ins/ins/AA, del/ins/GA and ins/ins/GG genotype ($P = 0.621$ and $P = 0.864$, respectively).

## Discussion

Obesity is recognized as low-grade inflammation characterized by an increase in circulating levels of CRP [1, 2]. Therefore, the inflammation seems a putative link to explain the mechanisms between obesity and obesity-related disorders [24]. However, the studies focused on the inflammation-related genetic structural alterations in obese patients are very limited and seeking for novel candidate genes is still a challenge.

Many researchers have pointed that variations within the *NFKB1* and *TLR2* genes could potentially influence the process of inflammation. According to Paepegaey *et al.*, distribution of CRP, a marker of systemic inflammation, in MO is considered as a model of extreme adipose tissue expansion. In obese patients, high CRP levels above

**Table 3** The distribution of genotype and allele frequencies of rs28362491 and rs5743708 polymorphisms in morbid obesity patients with subgroups and control group.

| Genotype/allele | Control, *n* (%) | Morbid obesity subgroups | | | | | |
|---|---|---|---|---|---|---|---|
| | | HI group *n* (%) | *P** | OR (95% CI) | LI group *n* (%) | *P** | OR (95% CI) |
| NFKB1 | | | | | | | |
| ins/ins | 73 (35) | 42 (49) | Ref. | | 46 (41) | Ref. | |
| del/ins | 108 (52) | 34 (40) | **0.0280** | 1.828 (1.064–3.140) | 48 (43) | 0.1719 | 0.7053 (0.427–1.165) |
| del/del | 26 (13) | 10 (11) | 0.3353 | 1.496 (0.6573–3.405) | 18 (16) | 0.7937 | 1.099 (0.5427–2.224) |
| ins/ins+del/ins/del/del | | | 0.4304 | 1.604 (0.632–4.070) | | 0.9793 | 1.092 (0.5019–2.375) |
| ins/ins/del/ins+del/del | | | **0.0204** | 1.887 (1.099–3.239) | | 0.2118 | 1.386 (0.8295–2.316) |
| ins allele frequency | 254 (61) | 118 (69) | Ref. | | 140 (63) | Ref. | |
| del allele frequency | 160 (39) | 54 (31) | 0.0968 | 1.376 (0.9432–2.009) | 84 (37) | 0.7611 | 1.073 (0.6827–1.685) |
| TLR2 | | | | | | | |
| GG | 144 (74) | 73 (100) | Ref. | | 86 (100) | Ref. | |
| GA | 39 (20) | 0 (0) | **<0.0001** | 40.18 (2.434–663.5) | 0 (0) | **<0.0001** | 47.29 (2.868–779.8) |
| AA | 11 (6) | 0 (0) | **0.0453** | 11.70 (0.679–201.4) | 0 (0) | **0.0273** | 13.77 (0.8007–236.7) |
| GG+GA/AA | | | 0.0832 | 9.213 (0.536–158.5) | | 0.0549 | 10.84 (0.6311–186.2) |
| GG/GA+AA | | | **<0.0001** | 51.37 (3.123–845.1) | | **<0.0001** | 60.46 (3.681–993.2) |
| G allele frequency | 327 (84) | 146 (100) | Ref. | | 172 (100) | Ref. | |
| A allele frequency | 61 (16) | 0 (0) | **<0.0001** | 55.02 (3.378–896.3) | 0 (0) | **<0.0001** | 64.79 (3.980–1055) |

Ref., reference.

**P* calculated by chi-square test as compared to controls.

Statistically significant values ($p < 0.05$) are marked in bold.

**Table 4** Determination of the genetic effects of rs28362491 and rs5743708 polymorphisms on MO.

| | $\chi^2$ | df | *P* value |
|---|---|---|---|
| TLR2-NFKB1 | | | |
| ins/ins – GG | 53.877 | 5 | **0.000** |
| ins/ins – GA | 18.758 | 1 | **0.000** |
| ins/ins – AA | 5.341 | 1 | **0.021** |
| del/ins – GG | 0.245 | 1 | 0.621 |
| del/ins – GA | 21.081 | 1 | **0.000** |
| del/del – GG | 0.029 | 1 | 0.864 |
| Overall statistics | | | |
| ins/ins – GG | 53.877 | 5 | **0.000** |

$\chi^2$, chi-square; OR, odds ratio; df, degrees of freedom.
del/ins+AA, del/del+GA and del/del+AA combined genotypes were excluded in table as none observed in the subjects or the size was not enough for the statistical analysis.
Statistically significant values ($p < 0.05$) are marked in bold.

20 mg/l would be related to fat mass expansion and adipose tissue inflammation. It is well established that CRP levels are increased in obesity, diminished during weight loss [25] and correlated with BMI [4].

Our data demonstrated that increased serum CRP levels could be related to promotor polymorphism of *NFKB1*. In this study, *NFKB1*-94 ins/del ATTG polymorphisms have been analysed in 213 patients suffering from MO and 200 healthy controls. According to our results, having ins/ins genotype and/or ins allele of rs28362491 has 1.58 and 1.35 times risk factors in patients with MO. In addition, in the case of CRP levels, the risk of ins/ins genotype in MO compared to the control group is increased 1.82 times of ≥20 mg/l versus <20 mg/l. Nonetheless, Stegger *et al.* [12] did not find any interaction between *NFKB1*-94 ins/del ATTG polymorphisms and general, abdominal or gluteofemoral obesity. We have reported the association of this polymorphism of *NFKB1* gene with many inflammatory diseases such as Hashimoto thyroiditis, Graves' disease and Behcet's disease [26] which was also investigated in many diseases such as coronary artery disease [27], Crimean-Congo haemorrhagic fever [28] and ulcerative colitis [6].

Recent studies have shown that the variant allele containing the deletion *NFKB1*-94 ins/delATTG promoter polymorphism leads to lower levels of the p50 subunit, and this affects both the availability of the anti-inflammatory p50/p50 NFKB homodimer and the pro-inflammatory p50/p65 NFKB heterodimer. However, the combined effect of relatively low levels of both p50/p50 and p50/p65 will be pro-inflammatory, as low p50 levels intuitively will affect the concentration of p50/p50 more than the concentration of p50/ p65. Furthermore, the p50/p50 homodimer blocks binding sites for the p65 subunit, and, thus, the level of the p50/p65 heterodimer will be relatively closer to normal, due to the concurrent low levels of p50/p50. CRP transcription is controlled by the p50 homodimer [6]. We have found higher levels of CRP protein in serum from carriers of the ins allele compared to del allele carriers, supporting the interpretation that the del allele causes depletion of p50 homodimer.

Another important result of our study is the relationship between *TLR2* Arg753Gln variations in MO and CRP levels. TLR2 is one of the most important innate immune receptors that play important roles in recognizing the pathogens and producing inflammatory cytokines [15]. Recently, the role of *TLR2* gene polymorphisms has been evaluated in systemic inflammatory diseases such as coronary artery disease [16, 17], children obesity [18] and inflammatory bowel disease (IBD) [29]. TLR2 receptors are elevated and associated with increasing BMI values in obese children [18]. Arg753Gln polymorphisms may not be associated with IBD in the Guangxi Zhuang population of China [29]. Studies in Turkish children indicate that the Arg753Gln *TLR2* polymorphism influences the speed of progression from infection to tuberculosis [30]. *TLR2* Arg753Gln genotype and 753Gln allele alone were associated with a higher risk of rheumatic fever, paediatric tuberculosis and vitiligo [31, 32], but there was no reported association with the risk of MO. TLR2 rs5743708 genotypes in the control group were not within the HWE. This is probably due to excess of heterozygotes as a result of genotyping error or control subjects being sampled from different ethnic groups. Our Arg753Gln genotyping data support the major role of Gln/Gln genotype and Gln allele in increased risk of MO and CRP levels.

rs28362491 CATTG allele (insertion) frequency was 68% for patient and 61% for control group in our study, which is similar among the populations such as 68% for African Caribbeans, 45% for African Americans, 61% for Han Chinese in Beijing and 55% for British in England. rs5743708 G allele was 100% for patient and 74% for control group in our study which is not divergent among the populations such as 100% for African Caribbeans, 100% for African Americans, 99% for Han Chinese in Beijing and 97% for British in England (1000 Genomes Browser – 01 August 2016).

Table 4 represents combined effects of polymorphisms including homozygous wild mutant and heterozygous genotypes of *NFKB1* and *TLR2*. It was claimed that ins/ins/GG, ins/ins/GA, ins/ins/AA, del/ins/GA and ins/ins/GG were significantly higher in the patient group versus the control group when compared with the combined del/ins/GG genotype and del/del/GG combined genotype ($P = 0.000$; $P = 0.000$; $P = 0.021$; $P = 0.000$; $P = 0.000$, respectively). This low risk was based on del allele from *NFKB1* rs28362491 in promoter region and G allele from *TLR2* rs5743708.

In conclusion, this study shows that polymorphisms within *NFKB1* and *TLR2* seem to be involved in the susceptibility to MO, and these polymorphisms may be valuable markers to predict the risk for the development of MO. Moreover, we also determined the association of SNPs

rs28362491 and rs5743708 in the context of CRP levels in MO as the existence of ins/ins and GG genotype is associated with high levels of CRP in MO. Detecting the ins allele of rs28362491 and 753Gln allele of rs5743708 with high level of CRP may offer a better treatment in the future.

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