Saudi Journal of Biological Sciences 28 (2021) 3548–3552

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Protective role of TIRAP functional variant against development of coronary artery disease

Mamoona Noreen [a,b,*], Muhammad Imran [c], Sher Zaman Safi [d], Muhammad Amjad Bashir [e], Sana Gul [f], Afrah Fahad Alkhuriji [g], Suliman Yousef Aloma [g], Hanan Mualla Alharbi [g], Muhammad Arshad [a,h]

[a] Atta ur Rehman School of Applied Biosciences (ASAB), National University of Sciences & Technology (NUST), H-12 sector, Islamabad, Pakistan
[b] Department of Zoology, The Women University Multan, Multan, Pakistan
[c] Department of Microbiology, University of Health Sciences, Lahore, Pakistan
[d] Interdisciplinary Research Center in Biomedical Materials (IRCBM), COMSATS Institute of Information Technology, Lahore, Pakistan
[e] Department of Plant Protection Faculty of Agricultural Sciences, Ghazi University, Dera Ghazi Khan, Pakistan
[f] Department of Biological Sciences National University of Medical Sciences (NUMS) Rawalpindi, Pakistan
[g] Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
[h] University of Education, Lahore, Pakistan

ARTICLE INFO

Article history:
Received 2 March 2021
Revised 8 March 2021
Accepted 8 March 2021
Available online 18 March 2021

Keywords:
TIRAP/MAL
Polymorphism
SNP
Coronary artery disease
Functional variant
Toll like Receptors

ABSTRACT

Coronary artery disease (CAD) is the leading cause of sudden death worldwide. Inflammation is proved to be an important player in development of the CAD. Inflammation is directly regulated by the Toll like receptors (TLRs). Susceptibility of CAD is influenced by genetic variations within TLRs and the proteins involved in its signaling cascade. The TIRAP/MAL {TIR domain containing adaptor protein / MyD88 (myeloid differentiation primary response gene 88) adaptor-like} exhibits maximum genetic variations of all adaptor proteins involved in TLR signaling cascade. Susceptibility to a number of diseases can be influenced due to presence of S180L single nucleotide polymorphism (SNP) of TIRAP/MAL. This study was conducted to investigate the functional role of this well characterized S180L polymorphism on susceptibility to CAD among Pakistani patients. A total of 146 Pakistani CAD patients and 147 controls were genotyped by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) and the data was analyzed by using 2-tailed Chi square (x^2) test. The p value $\leq$ 0.05 was considered to be significant.

Significantly high frequency of homozygous L180L genotype was observed among healthy subjects as compared to the CAD patients [24 (16%) vs 7 (5%); x^2 11.85; p = 0.003]. Moreover, the allele frequency of the minor allele; 180L was observed to be significantly higher among controls than the CAD patients, having same direction of association [156 (53%) vs 131 (45%); OR (95% CI) = 0.7198 (0.520–0.996); p < 0.05).

Our results indicate that protective effect of L180L; a coding variant of TIRAP/MAL against CAD is discernible.

1. Introduction

The most common form of cardiovascular diseases is coronary artery disease (CAD) or atherosclerosis which is also leading cause of sudden death (Thomas et al., 1988; Kalayoglu and Byrne, 1998). In CAD, the coronary arteries become hard and narrow resulting in obstructed blood flow to the myocardium. It is principally caused by a condition called atherosclerosis. Vascular oxidative stress, injury and invasion of mononuclear phagocytes may trigger the atherogenic inflammation (Inflammation, 2005; Zebrack and Anderson, 2002). Furthermore, not only oxidized and glycosylated products (e.g. modified lipoproteins) but also infectious agents like *Chlamydia pneumonia* (Shor et al., 1992); *Porphyromonas gingivalis* (Li et al., 2002) and Epstein-Barr virus (EBV), play key role in patho-

* Corresponding author at: Department of Zoology, The Women University Multan, Multan, Pakistan.
E-mail addresses: mamoona.noreen@gmail.com (M. Noreen), mi.bannu@yahoo.com (M. Imran), dr.szsafi@ciitlahore.edu.pk (S.Z. Safi), abashir@gudgk.edu.pk (M.A. Bashir), sana.gul@numspak.edu.pk (S. Gul), aalkhuriji@ksu.edu.sa (A.F. Alkhuriji), syalomar@ksu.edu.sa (S.Y. Aloma), halshami99@hotmail.com (H.M. Alharbi), marshad63@msn.com (M. Arshad).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.03.028
1319-562X/

genesis of the disease (Rupprecht et al., 2001). All these factors lead to chronic inflammation which is fundamental cause of atherosclerosis and CAD (Inflammation, 2005). Activation of the Toll like receptors (TLRs) Pathway also link inflammation, infection and pathogenesis of CAD. Evidence from diverse sources and experimental models has provided a wealth of information suggesting that TLRs could affect atherogenesis in multiple ways (Noreen et al., 2012). TLR2 and TLR4 seem to be mainly involved in progression of the disease. Up regulation of TLR2 and TLR4 has been reported to be associated with atherogenesis (Xu et al., 2001; Mizoguchi et al., 2007). Infection with *C. pneumonia* has been associated with atherosclerotic lesions (Shor and Phillips, 1999; Kuo et al., 1993). The symptoms of infection with *C. pneumoniae* are similar to those of atherogenesis, including increased lipid accumulation in macrophages (Kalayoglu and Byrne, 1998) and proliferation of human vascular smooth muscle cells (VSMCs) (Sasu et al., 2001). Role of *Porphyromonas gingivalis* (Gram-negative anaerobe) in development of atherosclerosis has been emphasized through human epidemiological studies (Gibson et al., 2006). Presence of EBV has also been reported to affect the outcome of CAD (Rupprecht et al., 2001) and progression of atherosclerosis (Liu et al., 2008; Schoneveld et al., 2005). Furthermore oxidized low density lipoprotein (LDL) (Steinberg et al., 1989) is known to activate and up-regulate the expression of TLR4 and thus resulting in secretion of the chemokine IL-8 (Walton et al., 2003).

Ligand binding to TLRs results in the production of many pro-inflammatory cytokines and chemokines which can be involved in the pathogenesis of atherogenesis (Michelsen et al., 2004). The TLR2 and TLR4 mediated MyD88-dependent downstream signaling pathway involves recruitment of the TIR (Toll/interleukin-1 receptor) domain containing adaptor protein (TIRAP) / MyD88 (myeloid differentiation primary response gene 88) adaptor-like (MAL) (Yamamoto et al., 2002; Mansell et al., 2004). The TIRAP/MAL is characterized to contain maximum number of genetic variations among all the proteins involved in TLR signaling cascade (Noreen and Arshad, 2015). The S180L / C539T (GenBank accession no. rs8177374) in TIRAP/MAL involved substitution of the serine by the leucine amino acid (aa) at position 180 of total 221 residues. It can alter NF-κB signaling thus protects against unnecessary inflammation (Khor et al., 2007). The strength of an inflammatory response is important enough to affect the disease outcome (Noreen et al., 2012). The TIRAP/MAL (S180L) polymorphism has been found to be associated with pneumococcal disease, bacteremia, tuberculosis (TB), malaria (Khor et al., 2007), sepsis (Hamann et al., 2009), systemic lupus erythematosus (SLE) (Castiblanco et al., 2008), rheumatoid arthritis (RA) (Castiblanco et al., 2008; Sheedy et al., 2008), axial spondyloarthritis (Cantaert et al., 2008) and chronic Chagas cardiomyopathy (CCC) (Ramasawmy et al., 2009). As the presence of the SNPs can affect susceptibility and progression of disease (Noreen et al., 2015) thus based on the crucial role of TIRAP/MAL polymorphism (S180L) in susceptibility to various diseases, the association of the polymorphism to CAD was investigated in Pakistani population.

2. Materials and methods

2.1. Subjects

Present study was conducted at Atta ur Rehman School of Applied Biosciences (ASAB), National University of Sciences & Technology (NUST), Pakistan. We included 146 informed consented patients of CAD [48 women (33%) and 98 men (67%)], aged between 38 and 82 years (mean age 58.23 ± 9.29 years) whom disease status was confirmed by coronary artery angiography from Hearts International Hospital, Rawalpindi, Pakistan. All participants were informed about the study and sample was taken after their consent . The age and sex matched control group of 149 individuals was selected with no evidence of CAD as proved after angiography. Ethnicity and age matched subjects were included in this study.

2.2. SNP genotyping

The peripheral blood samples were collected in 10 ml K_3 tubes containing ethylenediamine tetra-acetic acid (EDTA) (BD vacutainer, New Jersey, USA). The genomic DNA was isolated from peripheral blood using the DNA purification kit (Gentra Puregene, Hilden, Germany) as per manufacturer's protocol. The DNA of each sample was quantified using the PicoGreen dsDNA assay kit (Life Technologies, New York, USA). The TIRAP/MAL coding sequence was taken from Genbank and two sets of primers were designed using the software Primer3 (http://frodo.wi.mit.edu/). The difference in primers of both sets was of only one base at 3′ end of forward primers, while the reverse primer was quite identical in both the cases. The forward primer of first set (F1) had cytosine (C) at its 3′ end, which makes it complimentary to the region of DNA which encodes for serine at position 180 of the TIRAP/MAL protein while the forward primer of second set (F2) had thymine (T) at 3′ end, which enables this set of primers to amplify the region of DNA which will encode for leucine at position 180 in protein sequence.

Following primers were used to amplify the desired region of 926 bp.

Forward Primer F1: 5′-GGCTGCACCATCCCCCTGCTGTC-3′

Forward Primer F2: 5′-GGCTGCACCATCCCCCTGCTGTT-3′

Reverse primer R: 5′-AGTCCCCAAGCTCTCCATGGTCTTCTTAGG-3′

Another set of primers for IL-10 gene was designed as internal control to check validity of amplification process. Following set of primers was used to amplify the region of 412 bases.

Forward Primer: 5′-CCTAGGTCACAGTGACGTGG-3′

Reverse primer: 5′-GGTGAGCACTACCTGACTAGC-3′

Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) was performed to discriminate among templates which differ by a single nucleotide residue. 3.5 µl of the sample DNA (50 ng/µl), 2 µl (10X) of PCR buffer (Fermentas, Canada), 1.5 µl of 2 mM deoxyribonucleotide triphosphates (dNTPs) mixture (MBI Fermentas, Canada), 1.6 µl of 25 mM magnesium chloride ($MgCl_2$) (MBI Fermentas, Canada), 1 µl of each forward primer (10 pm/µl) either F1 or F2 and forward primer of internal control, 1 µl of each of reverse primers (10 pm/µl) and 0.4 µl Taq DNA polymerase (MBI Fermentas, Canada) was mixed in 7.0 µl neuclease free water to prepare 20 µl PCR reaction mixture.

The reaction mixture was subjected to the thermocycling starting with template denaturation for 5 min at 94 °C followed by 35 amplification cycles. Each cycle consisted of 3 steps; 45 s at 94 °C for DNA denaturation, 30 s at 63 °C for primers annealing to respective template strands and 80 s at 72 °C for extension of the complementary DNA strand from each primer. Finally the reaction mixture went through 72 °C for 10 min for Taq polymerase to synthesize if any unextended strands were left. The PCR was performed using thermal cycler (Applied Biosystem, USA). The PCR products were subjected to electrophoresis in a 2% agarose gel and stained with the ethidium bromide (Fig. 1). After electrophoresis, the amplified product was detected and photographed using UV Transilluminator (Biometra, Germany) and using gel documentation system (Wealtec, USA) respectively.

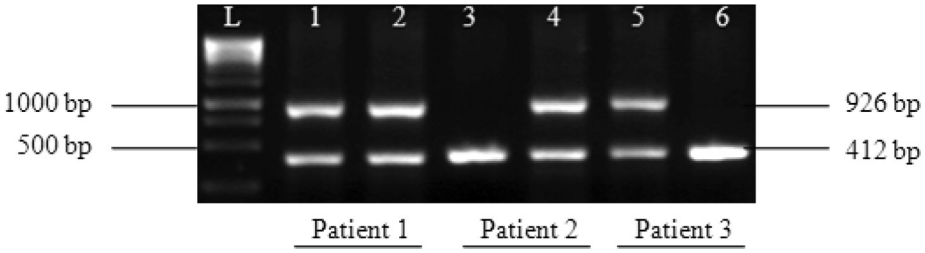

Fig. 1. 2 % Agarose Gel showing genotypes of three patients of CAD according to the TIRAP/MAL polymorphism (S180L). The lane 2, 4, 6 contain amplified products of 1st, 2nd and 3rd patients respectively by using primers (F1+R) and positive control. The lane 1, 3 and 5, contain amplified products of 1st, 2nd and 3rd patients respectively by using primers (F2+R) and positive control. Amplification of desired region (926 bp) in lane 1, 2, 4 and 5 and absence of bands in lane 3 and 6 show that 1st patient is heterozygous (S180L), 2nd patient is homozygous (L180L) and 3rd patient is homozygous (S180S) for TIRAP/MAL polymorphism (S180L).

2.3. Statistical analysis

Allelic and genotypic frequencies were manually counted and the polymorphism was checked for deviation from Hardy Weinberg equilibrium (HWE). Prism (version 5.0; GraphPad) was used for statistical analysis. The allelic and genotypic associations with disease groups and control were analyzed by use of the Chi square (x^2) test and 2-tailed Fisher's exact tests. OR with 95% confidence intervals (CI) was also calculated. The p value $\leq$ 0.05 was taken to be significant.

3. Results

3.1. Genotypic frequencies in CAD patients

It was found that the frequency of homozygous S180S genotype in CAD patients was significantly higher than the healthy individuals [46 (32%) vs 27(18%) p = 0.02] in Pakistani population. The heterozygous S180L genotype was found in 76 (52%) CAD patients and 86 (58%) members of control group. The homozygous L180L genotype was more frequent 36 (24%) among healthy subjects as compared to 24 (16%) CAD patients (Table 1, Fig. 2).

3.2. Allelic frequencies in CAD patients

When allelic frequencies of CAD patients were compared with that of healthy individuals, the frequency of 180S allele was found to be higher among CAD patients 168 (58%) as compared to healthy individuals 140 (47%). In healthy individuals 180L allele was significantly more frequent 158 (53%) than diseased ones i.e 124 (42%) (Table 2). Preventive effect of 180L allele with susceptibility to CAD was significantly evident [OR (95% CI) = 1.53 (1.11 to 2.12); p = 0.01)] (Fig. 3).

4. Discussion

During last few decades, the medical geneticists have been eagerly interested to unravel the role of genetic variations on pathogenesis and susceptibility of diseases. Genes involved in pathogen recognition and signaling pathways are of prime importance in this concern. Variations in the inflammation associated genes significantly affect the immune response and pathogenesis of the diseases (Noreen et al., 2012).

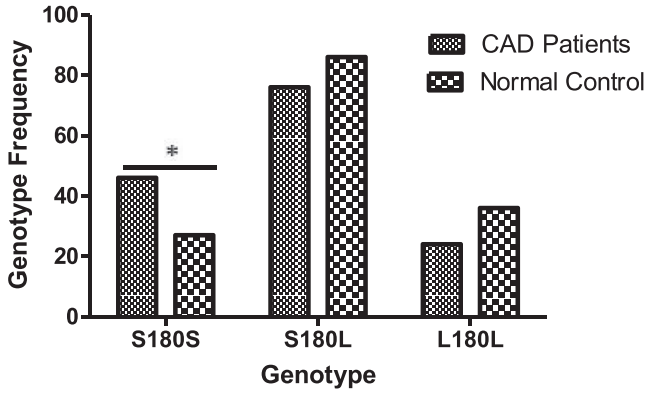

Fig. 2. Genotype frequency in CAD patients and controls and their susceptibility to CAD.

As CAD is characterized to be the root cause of sudden casualties, (Thomas et al., 1988) thus, it was also important to elucidate the involvement of TIRAP/MAL (S180L) with susceptibility to the CAD. Accumulation of atheromatous plaques within coronary arteries causes CAD. In the beginning, endothelial injuries appear and then progress to advanced and complicated pathological lesions (Inflammation, 2005). The CAD is not merely a passive infiltration of the lipids but is an active and continuous inflammatory process, (Zebrack and Anderson, 2002). It has also been reported that the pathological determinants involved in development and destabilization of plaque have strong implication with TLRs and ultimately regulation of the immune system.

Progression of atherosclerotic lesions may be negotiated by ligation of TLR2 (Schoneveld et al., 2005). Link between TLR4 and atherogenesis was indicated not only by *in vitro* up regulation of TLR4 in human macrophages by oxidized LDL but also by preferential expression of TLR4 by macrophages in lipid rich atherosclerotic lesions of mice as well as humans (Xu et al., 2001). Activation of TLR4 results in expression of many pro-inflammatory cytokines,

Table 1
Distribution of genotypic frequencies of TIRAP/MAL polymorphism (S180L) in healthy individuals and CAD patients.

Genotype				Chi Square Statistics	
	S180S n (%)	S180L n (%)	L180L n (%)	x^2 Value	p Value
CAD Patients (N = 146)	46 (32)	76 (52)	24 (16)	7.93	0.02
Normal Control (N = 149)	27 (18)	86 (58)	36 (24)		

Note: N = total number of individuals; n = number of individuals. The p $\leq$ 0.05 was considered to be significant.

Table 2
Allelic frequencies of TIRAP/MAL polymorphism (S180L) in healthy individuals and CAD patients.

Allele frequency			Fisher's exact test	
	180S n (%)	180L n (%)	OR (95% CI)	p Value
CAD Patients N = 292	168 (58)	124 (42)	1.53 (1.11 to 2.12)	0.01
Normal Control N = 298	140 (47)	158 (53)		

Note: N = total number of alleles; n = number of allele; OR = odds ratio; CI = confidence interval. The $p \leq 0.05$ was considered to be significant.

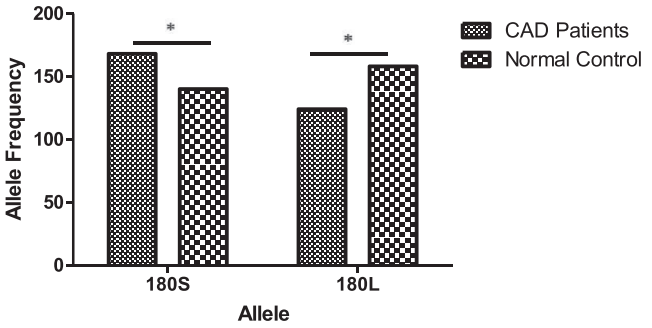

Fig. 3. Distribution of allelic frequency in CAD patients and controls. More frequent presence of 180L among healthy individuals indicates its protective effect against CAD.

which are key players in atherogenesis (Walton et al., 2003). So, susceptibility to CAD may be affected by genetic differences which ultimately influence the nature and strength of an inflammatory response (Noreen et al., 2012).

In this study we found that, TIRAP/MAL coding variant (S180L) is associated with susceptibility to CAD, in Pakistani population. In patients of CAD, there was significantly higher frequency of homozygous S180S genotype (46 (32%) vs 27(18%) p = 0.02) in comparison with controls. The heterozygous S180L genotype was less frequent among patients (52% vs 58%) but the difference was non-significant (p>0.05). On the other hand, homozygous L180L genotype was more frequent among the healthy subjects as compared to the CAD patients (24% vs 16%) (Table 1). The difference in genotypic frequencies reveals significant association of the homozygous S180S genotype with susceptibility to CAD (x^2 7.93; p = 0.02) and shows the protective effect of L180L genotype against the disease (Fig. 2).

When allelic frequencies of CAD patients were compared with that of healthy individuals, the frequency of 180S allele was found to be more among CAD patients as compared to healthy individuals (58 vs 47 per cent). The 180L allele was also more frequent among healthy individuals (53 vs 42 per cent) (Table 2). The results indicated significant association of both the alleles with susceptibility to CAD [OR (95% CI) = 1.53 (1.11 to 2.12); p= <0.01)] (Fig. 3). The scarcity of such studies in the World makes it difficult to assume that whether this polymorphism is linked to CAD among other populations or not. However, the occurrence of significantly high percentage of homozygous mutant genotype (L180L) and minor allele (180L) in the healthy subjects as compared to the CAD patients shows its protective role in the pathogenesis of CAD. The allelic and genotypic frequencies of S180L in Pakistani population are different from other studies (Khor et al., 2007; Hamann et al., 2009). These disparities may be explained on the basis of differences in ethnicity. The heterozygous genotype has been reported to have protective association against diseases due to optimal production of pro inflammatory cytokines as compared to wild type homozygous genotype (Khor et al., 2007). The prevalence of high percentage of the heterozygous genotype in healthy individuals may be hypothesized due to its protective role.

There are several plausible evidences provided by the molecular mechanisms, explaining the role of TIRAP/MAL polymorphism (S180L) in decreased signal transduction efficiency which leads to impaired cytokine production. Dunne et al. has reported the interaction of wild type TIRAP/MAL with TLR2 (Dunne et al., 2003) and Khor et al. demonstrated that TLR2 was unable to recruit variants thus making the signaling process defective. The variant form of TIRAP/MAL was also observed to inhibit the ability of wild TIRAP/MAL to activate NF-κB (Khor et al., 2007).

Thus the functional studies of TIRAP/MAL polymorphism (S180L) have elegantly proved that the wild type TIRAP/MAL is implicated to produce high levels of pro inflammatory cytokines while the mutant type may have the contrasting effect. As the immunopathology of CAD is affected by production of pro-inflammatory cytokines, their impaired production in homozygous mutant (L180L) individuals may be important in hypothesizing the protective role against the CAD in our results. As the population included in study is small thus same type of studies with large population cohort and in different ethnic groups in future may open further avenues to confirm the role of this SNP in CAD.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at the King Saud University for funding this work through research group no.RG-1441-356.

References

Cantaert, T., Stone, M.A., ter Borg, M., Mogg, R., De Vries, N., Wilson, A.G., et al., 2008. A functional polymorphism of TIR-domain-containing adaptor protein is not associated with axial spondyloarthritis. Ann. Rheum. Dis. 67 (5), 720–722. Epub 2007/12/13. doi: ard.2007.082784 [pii] 10.1136/ard.2007.082784. PubMed PMID: 18073264.

Castiblanco, J., Varela, D.C., Castano-Rodriguez, N., Rojas-Villarraga, A., Hincapie, M. E., Anaya, J.M., 2008. TIRAP (MAL) S180L polymorphism is a common protective factor against developing tuberculosis and systemic lupus erythematosus. Infect. Genet. Evol. 8 (5), 541–544. Epub 2008/04/18. doi: 10.1016/j. meegid.2008.03.001 S1567-1348(08)00046-4 [pii]. PubMed PMID: 18417424.

Dunne, A., Ejdeback, M., Ludidi, P.L., O'Neill, L.A., Gay, N.J., 2003. Structural complementarity of Toll/interleukin-1 receptor domains in Toll-like receptors and the adaptors Mal and MyD88. J. Biol. Chem. 278 (42), 41443–41451. Epub 2003/07/31. doi: 10.1074/jbc.M301742200 M301742200 [pii]. PubMed PMID: 12888566.

Gibson, F.C., 3rd, Yumoto, H., Takahashi, Y., Chou, H.H., Genco, C.A., 2006. Innate immune signaling and Porphyromonas gingivalis-accelerated atherosclerosis. J. Dent. Res., 85, 2, 106–121. Epub 2006/01/26. doi: 85/2/106 [pii]. PubMed PMID: 16434728.

Hamann, L., Kumpf, O., Schuring, R.P., Alpsoy, E., Bedu-Addo, G., Bienzle, U., et al., 2009. Low frequency of the TIRAP S180L polymorphism in Africa, and its potential role in malaria, sepsis, and leprosy. BMC Med. Genet. 10, 65. Epub 2009/07/16. doi: 10.1186/1471-2350-10-65 1471-2350-10-65 [pii]. PubMed PMID: 19602285; PubMed Central PMCID: PMC2717080.

Hansson, G.K., 2005. Inflammation, atherosclerosis, and coronary artery disease. N. Engl. J. Med. 352 (16), 1685–1695. Epub 2005/04/22. doi: 352/16/1685 [pii] 10.1056/NEJMra043430. PubMed PMID: 15843671.

Kalayoglu, M.V., Byrne, G.I., 1998. Induction of macrophage foam cell formation by Chlamydia pneumoniae. J. Infect. Dis. 177 (3), 725–729. Epub 1998/03/14 PubMed PMID: 9498454.

Khor, C.C., Chapman, S.J., Vannberg, F.O., Dunne, A., Murphy, C., Ling, E.Y., et al., 2007. A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet. 39 (4), 523–528. Epub 2007/02/27. doi: ng1976 [pii] 10.1038/ng1976. PubMed PMID: 17322885; PubMed Central PMCID: PMC2660299.

Kuo, C.C., Gown, A.M., Benditt, E.P., Grayston, J.T., 1993. Detection of Chlamydia pneumoniae in aortic lesions of atherosclerosis by immunocytochemical stain. Arterioscler. Thromb. 13 (10), 1501–1504. Epub 1993/10/01 PubMed PMID: 7691166.

Li, L., Messas, E., Batista Jr., E.L., Levine, R.A., Amar, S., 2002. Porphyromonas gingivalis infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. Circulation. 105 (7), 861–867. Epub 2002/02/21 PubMed PMID: 11854128.

Liu, X., Ukai, T., Yumoto, H., Davey, M., Goswami, S., Gibson, F.C., 3rd, et al., 2008. Toll-like receptor 2 plays a critical role in the progression of atherosclerosis that is independent of dietary lipids. Atherosclerosis. 196, 1, 146–154. Epub 2007/05/01. doi: S0021-9150(07)00188-8 [pii] 10.1016/j.atherosclerosis.2007.03.025. PubMed PMID: 17466307; PubMed Central PMCID: PMC2243224.

Mansell, A., Brint, E., Gould, J.A., O'Neill, L.A., Hertzog, P.J., 2004. Mal interacts with tumor necrosis factor receptor-associated factor (TRAF)-6 to mediate NF-kappaB activation by toll-like receptor (TLR)-2 and TLR4. J. Biol. Chem. 279 (36), 37227–37230. Epub 2004/07/13. doi: 10.1074/jbc.C400289200 C400289200 [pii]. PubMed PMID: 15247281.

Michelsen, K.S., Doherty, T.M., Shah, P.K., Arditi, M., 2004. TLR signaling: an emerging bridge from innate immunity to atherogenesis. J. Immunol. 173 (10), 5901–5907. Epub 2004/11/06. doi: 173/10/5901 [pii]. PubMed PMID: 15528321.

Mizoguchi, E., Orihara, K., Hamasaki, S., Ishida, S., Kataoka, T., Ogawa, M., et al., 2007. Association between Toll-like receptors and the extent and severity of coronary artery disease in patients with stable angina. Coron. Artery Dis. 18 (1), 31–38. Epub 2006/12/19. doi: 10.1097/MCA.0b013e328010a474 00019501-200702000-00005 [pii]. PubMed PMID: 17172927.

Noreen, M., Arshad, M., 2015. Association of TLR1, TLR2, TLR4, TLR6, and TIRAP polymorphisms with disease susceptibility. Immunol. Res. 62 (2), 234–252. Epub 2015/03/19. doi: 10.1007/s12026-015-8640-6. PubMed PMID: 25784622.

Noreen, M., Shah, M.A., Mall, S.M., Choudhary, S., Hussain, T., Ahmed, I., et al., 2012. TLR4 polymorphisms and disease susceptibility. Inflamm. Res. 61 (3), 177–178. Epub 2012/01/27. doi: 10.1007/s00011-011-0427-1. PubMed PMID: 22277994.

Noreen, M., Murad, S., Khan, H., 2015. In Silico Analysis of SNPs in Coding Region of Human c-Myc Gene. Pakistan. J. Zool. 47 (5).

Ramasawmy, R., Cunha-Neto, E., Fae, K.C., Borba, S.C., Teixeira, P.C., Ferreira, S.C., et al., 2009. Heterozygosity for the S180L variant of MAL/TIRAP, a gene expressing an adaptor protein in the Toll-like receptor pathway, is associated with lower risk of developing chronic Chagas cardiomyopathy. J. Infect. Dis. 199 (12), 1838–1845. Epub 2009/05/22. doi: 10.1086/599212. PubMed PMID: 19456234.

Rupprecht, H.J., Blankenberg, S., Bickel, C., Rippin, G., Hafner, G., Prellwitz, W., et al., 2001. Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. Circulation. 104 (1), 25–31. Epub 2001/07/04 PubMed PMID: 11435333.

Sasu, S., LaVerda, D., Qureshi, N., Golenbock, D.T., Beasley, D., 2001. Chlamydia pneumoniae and chlamydial heat shock protein 60 stimulate proliferation of human vascular smooth muscle cells via toll-like receptor 4 and p44/p42 mitogen-activated protein kinase activation. Circ. Res. 89 (3), 244–250. Epub 2001/08/04 PubMed PMID: 11485974.

Schoneveld, A.H., Oude Nijhuis, M.M., van Middelaar, B., Laman, J.D., de Kleijn, D.P., Pasterkamp, G., 2005. Toll-like receptor 2 stimulation induces intimal hyperplasia and atherosclerotic lesion development. Cardiovasc. Res. 66 (1), 162–169. Epub 2005/03/17. doi: S0008-6363(04)00588-7 [pii] 10.1016/j.cardiores.2004.12.0PubMed PMID: 15769459.

Sheedy, F.J., Marinou, I., O'Neill, L.A., Wilson, A.G., 2008. The Mal/TIRAP S180L and TLR4 G299D polymorphisms are not associated with susceptibility to, or severity of, rheumatoid arthritis. Ann. Rheum. Dis. 67 (9), 1328–1331. Epub 2008/01/09. doi: 10.1136/ard.2007.083337 ard.2007.083337 [pii]. PubMed PMID: 18180278.

Shor, A., Kuo, C.C., Patton, D.L., 1992. Detection of Chlamydia pneumoniae in coronary arterial fatty streaks and atheromatous plaques. S Afr. Med. J. 82 (3), 158–161. Epub 1992/09/01 PubMed PMID: 1519134.

Shor, A., Phillips, J.I., 1999. Chlamydia pneumoniae and atherosclerosis. JAMA 282 (21), 2071–2073. Epub 1999/12/doi: jct90028 [pii]. PubMed PMID: 10591391.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., Witztum, J.L., 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N. Engl. J. Med. 320 (14), 915–924. Epub 1989/04/06. doi: 10.1056/NEJM198904063201407. PubMed PMID: 2648148.

Thomas, A.C., Knapman, P.A., Krikler, D.M., Davies, M.J., 1988. Community study of the causes of "natural" sudden death. BMJ 297 (6661), 1453–1456. Epub 1988/12/03. PubMed PMID: 3147014; PubMed Central PMCID: PMC1835183.

Walton, K.A., Hsieh, X., Gharavi, N., Wang, S., Wang, G., Yeh, M., et al., 2003. Receptors involved in the oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine-mediated synthesis of interleukin-8. A role for Toll-like receptor 4 and a glycosylphosphatidylinositol-anchored protein. J. Biol. Chem. 278 (32), 29661–29666. Epub 2003/06/05. doi: 10.1074/jbc.M300738200 M300738200 [pii]. PubMed PMID: 12777373.

Xu, X.H., Shah, P.K., Faure, E., Equils, O., Thomas, L., Fishbein, M.C., et al., 2001. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. Circulation. 104 (25), 3103–3108. Epub 2001/12/19 PubMed PMID: 11748108.

Yamamoto, M., Sato, S., Hemmi, H., Sanjo, H., Uematsu, S., Kaisho, T., et al., 2002. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. Nature 420 (6913), 324–329. Epub 2002/11/26. doi: 10.1038/nature01182 nature01182 [pii]. PubMed PMID: 12447441.

Zebrack, J.S., Anderson, J.L., 2002. The role of inflammation and infection in the pathogenesis and evolution of coronary artery disease. Curr. Cardiol. Rep. 4 (4), 278–288. Epub 2002/06/08 PubMed PMID: 12052267.