

## Effect of Genetic Polymorphisms in CD14 and TLR4 on Cardiomyopathy

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### Abstract

**Background:** We investigated polymorphisms of immune related genes (CD14 and TLR4) and explore the associations of these variants and risk of CM.

**Method:** We detected SNPs of CD14 (-159C>T) and TLR4 (299 A>G) using the polymerase chain reaction (PCR) with peripheral blood samples from 141 patients with CM and 198 healthy people, further analyzing their relations with the risk of CM.

**Result:** The CT genotype of CD14 gene was found to be associated with CM and 1.84 folds increased risk to CM when compared to CC genotype (OR 1.84, 95% CI 1.18–2.88, P=0.0051). Heterozygotes (AG) of TLR4 gene were found to be predominant in the CM group when compared to controls (50.4%, 38.4% respectively, P=0.0081) with 1.79 folds increased risk for CM, which was statistically significant (OR 3.79, 95% CI 1.15–2.80, P=0.0081).

**Conclusion:** Polymorphism in CD14 and TLR4 gene is associated with an increased risk of CM. The combined effects of polymorphisms within innate immune genes of CD14 and TLR4 may contribute to a high risk of CM.

**Keywords:** CM; CD14; TLR4; Polymorphisms

### Introduction

Cardiovascular diseases (CVD's) remain one of the major reasons of mortality universally, although since last two decades cardiovascular death rates have reduced in several developed countries. CVD are an international cause of death and accounts up to 31% of deaths alone, in India. They are the foremost reason for morbidity and mortality together in rural and urban India. The burden of CVD and its hazard factors in India makes it intended for a sound public health advancement to eradicate the epidemic. One such cardiovascular anarchy is Cardiomyopathy, which is a heterogeneous group of diseases linked with myocardial dysfunction and exhibiting improper ventricular hypertrophy or dilatation owing to an array of causes which are generally genetic. The changes in the environmental, hereditary conditions and mostly the innate immune response alterations play a significant role in pathogenesis of cardiomyopathy [1]. It have been hypothesized that the immune response is not steady all through the population and diverse motifs of immune response are measured to be associated through the polymorphisms in the immune genes. Distorted expression of innate immunity genes causes an unevenness in pro- and anti-inflammatory cytokines, which has been involved in the development and unremitted course of cardiac dysfunction [1,2]. The cytokine macrophage migration inhibitory factor (MIF), CD14, and Toll-like receptor 4 (TLR4) are interlinked components through evidently definite roles in immunologic and inflammatory pathways [3].

The major receptor for LPS is the CD14 receptor, a pattern identification molecule of the innate immune system [4]. CD14 has been concerned in monocyte activation [5] leucocyte-endothelial cell interactions [6], and regulation of programmed cell death (apoptosis) in both monocytes and endothelial cells [7,8], fundamental processes in the progress of atherosclerosis and its complications. The major significant CD14 co-receptor, TLR4 is in charge for activating intracellular signalling pathways. Functional polymorphisms in recent times recognized in both CD14 promoter and TLR4 genes [9,10] have been linked through acute coronary events [11,12], signifying that the strength of the genetically determined inflammatory response against pathogens or their antigens might contain a key function in determining the level of atherogenesis and consequent clinical result. This current study is trying to assess the clinical implications of the CD14/TLR4 polymorphisms in the progression of cardiomyopathy.

### Materials and Method

#### Patients and study design

One hundred and forty one patients (104 males and 67 females) with Cardiomyopathy were included in the present study. The cohort for the present study was selected from the symptomatic subjects at the Department of Cardiology, Deccan College of Medical Sciences Hyderabad, India. Exclusion criteria are includes patients with concomitant chronic inflammatory diseases such as arthritis, upper and lower GI disorder etc. Information on the symptoms, disease, duration, and history of any disease that could affect the study

outcome was obtained from each subject. One hundred and ninety eight healthy volunteers (131 males and 82 females) who served as case control were recruited in the present study. The study protocol was approved by the Institutional Ethics Committee, Deccan College of Medical Sciences Hyderabad, India. All study participants were asked to provide a written and signed consent to be part of this study protocol.

### Genotyping

Two millilitre (2 mL) of peripheral venous blood was collected by venepuncture from all the subjects. DNA was isolated from 1000 µL whole blood using a commercially available kit (Bioserve Biotechnology Pvt. Ltd., Hyderabad, India). Genotyping of CD14-159C>T polymorphisms was performed using tetra primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) as described previously [13] with minor modifications (annealing temperature 59°C for CD14). Polymorphism in the TLR4 (299 A>G) was performed by allele-specific amplification as described previously [14] (Table 1).

### In silico analysis (Online tools for in silico analysis)

Secondary protein structure prediction was performed by: PSIPRED <http://bioinf4.cs.ucl.ac.uk:3000/psipred/>

### Statistical analysis

Odds ratios, with 95% confidence intervals were calculated to compare allele and genotype frequencies. The extent of linkage disequilibrium (LD) was expressed in terms of the maximum likelihood estimate of disequilibrium, D'. Statistical analysis was carried out using SNPstats software, available online ([www.bioinfo.iconcologia.net/SNPstats](http://www.bioinfo.iconcologia.net/SNPstats)) and Haploview software. For all tests, significance level was set as P<0.05.

### Results

The frequency of 'T' allele was found to be predominant in CM group compared to controls (0.29 vs. 0.19 respectively).

Allele	Controls		Patients	
	Number	Frequency	Number	Frequency
C	320	0.81	200	0.71
T	76	0.19	82	0.29

**Table 1:** CD14 allele frequency distribution in controls and CM patients.

Model	Genotype	Controls	Patients	OR (95% CI)	P-value
Codominant	C/C	125 (63.1%)	66 (46.8%)	1.00	0.0051
	C/T	70 (35.4%)	68 (48.2%)	1.84 (1.18-2.88)	
	T/T	3 (1.5%)	7 (5%)	4.42 (1.11-17.65)	

Dominant	C/C	125 (63.1%)	66 (46.8%)	1.00	0.0028
	C/T-T/T	73 (36.9%)	75 (53.2%)	1.95 (1.25-3.02)	
Recessive	C/C-C/T	195 (98.5%)	134 (95%)	1.00	0.066
	T/T	3 (1.5%)	7 (5%)	3.40 (0.86-13.37)	
Overdominant	C/C-T/T	128 (64.7%)	73 (51.8%)	1.00	0.018

**Table 2:** Odd's risk estimation of the CD14 genotype in CM compared to control subjects.

The CT genotype was found to be associated with 1.84 folds increased risk to CM when compared to CC genotype (OR 1.84, 95% CI 1.18–2.88, P=0.0051). Based on the dominant model, combination of CT+TT genotypes were also observed to be associated with high risk for CM (OR 1.95, 95% CI 1.25–3.02, P=0.0028). Based on the recessive model TT genotype did not shown any statistical significance when compared to the combination of CC+CT genotype (OR 3.40, 95% CI 0.86–13.37, P=0.066). Whereas in over dominant model CT (Compared with CC+TT genotype) genotype was also observed to be associated with high risk to CM (OR 1.70, 95% CI 1.10–2.65, P=0.018). Further strengthening the association of 'T' allele with CM manifestation (Table 2).

Allele	Controls		Patients	
	Number	Frequency	Number	Frequency
A	310	0.78	193	0.68
G	86	0.22	89	0.32

**Table 3:** TLR4 allele frequency distribution in controls and CM.

The frequency of 'G' allele was found to be predominant in CM group when compared to controls (0.32 vs. 0.22 respectively) (Table 3).

Heterozygotes (AG) were found to be predominant in the CM group when compared to controls (50.4%, 38.4% respectively, P=0.0081) with 1.79 folds increased risk for CM, which was statistically significant (OR 3.79, 95% CI 1.15–2.80, P=0.0081) (Table 4). Based on the dominant model, combination of AG+GG genotypes were also observed to be associated with high risk for CM (OR 1.89, 95% CI 1.22–2.93, P=0.004). In recessive model the GG genotype (compared with AA +AG) did not reveal any risk to CM (OR 2.63, 95% CI 0.86–8.03, P=0.081) (Table 4). Whereas in overdominant model for AG (compared with AA+GG genotype) genotype was found to be associated with a 1.63 folds increased risk for CM (OR 1.63, 95% CI 1.05–2.52, P=0.028), further confirming the risk of 'G' allele in CM.

Model	Genotype	ID=0	ID=1	OR (95% CI)	P-value
Codominant	A/A	117 (59.1%)	61 (43.3%)	1.00	0.0081
	A/G	76 (38.4%)	71 (50.4%)	1.79 (1.15-2.80)	
	G/G	5 (2.5%)	9 (6.4%)	3.45 (1.11-10.75)	

Dominant	A/A	117 (59.1%)	61 (43.3%)	1.00	0.004
	A/G-G/G	81 (40.9%)	80 (56.7%)	1.89 (1.22-2.93)	
Recessive	A/A-A/G	193 (97.5%)	132 (93.6%)	1.00	0.081
	G/G	5 (2.5%)	9 (6.4%)	2.63 (0.86-8.03)	
Overdominant	A/A-G/G	122 (61.6%)	70 (49.6%)	1.00	0.028
	A/G	76 (38.4%)	71 (50.4%)	1.63 (1.05-2.52)	

**Table 4:** Odd's risk estimation of the TLR4 genotype in CM compared to control subjects.

Haplotype analysis is believed to be a more informative approach in strengthening the genetic influence on disease manifestation than testing for individual genotypes, hence haplotypes were constructed based on the two polymorphisms and analysed for the possible association with CM.

	CD14	TLR4	Freq	OR (95% CI)	P-value
1	C	A	0.7295	1.00	---
2	T	G	0.2207	2.02 (1.31-3.12)	0.0017
3	C	G	0.0375	1.39 (0.77-2.52)	0.28
4	T	A	0.0124	1.72 (0.47-6.29)	0.41

**Table 5:** Haplotype association with respect to various repair genes.

Of the all haplotypes obtained (Table 5), one haplotype carrying the recessive allele of CD14 and TLR4 polymorphism, TG was found to be significantly predominant in the disease group. The TG haplotype was found to be predominant in CM than controls with a 2.02 fold significant increase, (OR 2.02, 95% CI 1.31–3.12, P-0.0017) whereas the other haplotypes did not show any significance statistically. Hence TG haplotype could be the risk haplotype for CM, with the 'T' and 'G' alleles of CD14 and TLR4 respectively contributing significant risk to CM.

### Secondary Protein Structure Predictions

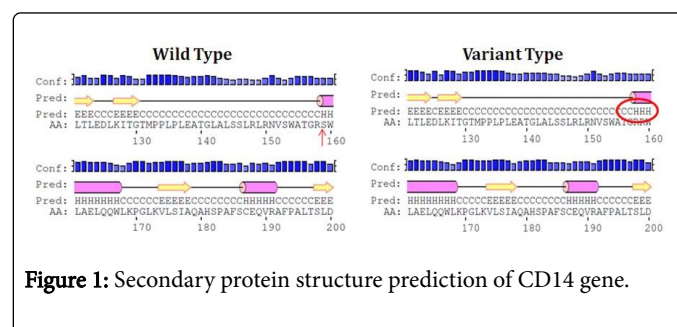
The variant sequence was subjected to PSIPRED online tool to predict the effect of the variation on the secondary protein structure. Secondary protein structures for the wild and the variant types.

The secondary structure prediction revealed the presence of a polymorphism which resulted in increased length of the helix in the variant type which may have an effect on protein folding and structure.

The secondary structure prediction revealed the presence of a polymorphism which resulted in removal of the helix in the variant type which may have an effect on protein folding and structure (Figure 1).

### Discussion

In the current study, we deliberated two polymorphisms (299 A>G and -159C>T) positioned in the TLR-4 and CD14 genes correspondingly in 141 patients with CM and 198 healthy controls. In humans, both polymorphisms influence TLR-4 and CD14 extracellular domain and individuals that have these polymorphisms are hyporesponsive to numerous ligands [14-16]. In this study, we noted that the allocation of the two polymorphisms were dissimilar in patients with CM and healthy controls. The connection of the TLR-4 and CD 14 genes polymorphisms with various diseases in diverse populations is notorious with positive and negative results.

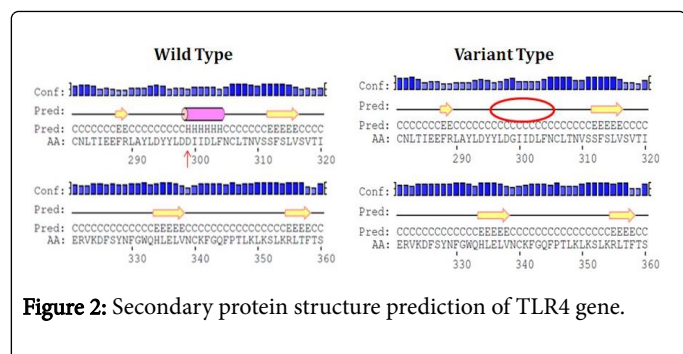


**Figure 1:** Secondary protein structure prediction of CD14 gene.

Our study has discovered that 299AG genotype of TLR4 gene found to be related in the CM group while compared to controls with 1.79 folds greater risk for CM. Robert et al. [17] found that association among the D299G polymorphism and threat of future MI or stroke which is analogous to our data. Hernesniemi et al. [18] reported that 299Gly allele linked with elevated carotid artery elasticity in a cohort of 2201 young adults. 299Gly allele carriers have extensively high carotid arterial observance, measured in enhance of luminal diameter percentage in response to blood pressure [18]. But, Martínez-Ríosa et al. [19] reported that there were no connections between TLR-4 gene polymorphisms and the cardiovascular risk factors in Caucasian population [19].

Furthermore, Zee et al. reported that the Asp299Gly polymorphism of TLR4 gene is not related through hazard of occurrence for myocardial infarction and stroke in 695 patients and 695 individuals control in a US population [20].

The CD14 receptor is well thought-out to be a monocyte activation marker and together augmented density of mCD14 and serum concentrations of sCD14 have been reported in patients with acute coronary syndromes [21,22]. Consequently, the reported connection among CD14 promoter polymorphism and the risk of MI may perhaps be a sign of a real susceptibility of patients who bear the T allele however it can also reveal either linkage disequilibrium or be a possibility finding [23]. These results are in exact consistency with several earlier studies supporting the roles of CD14-159C>T and TLR4-299A>G polymorphisms in rising susceptibility to Chlamydia pneumoniae infection [24] subgingival periodontopathogens [25], and Behcet's disease in a Korean population [26].



**Figure 2:** Secondary protein structure prediction of TLR4 gene.

The protein secondary structure forecast exposed enlarged length of the helix in CD14 gene and exclusion of helix in TLR4 gene (Figure 2), ultimately disturbing the protein folding. Even though the functional importance of this difference is not identified, a probable pathogenic cause was predicted. Ever since this difference was noted to be statistically important in the patient group compared to the controls, the likelihood of this difference in the pathogenicity of the disease under the influence of other genes and environmental factors cannot be ruled out. The reasons for the discrepancy in outcome wants an in detail investigation of the follow-up of events that leads to CM.

The Asp299Gly polymorphisms of TLR gene and -159C>T in CD14 gene were found to be linked through susceptibility of CM in our study population. Considering every part of this information collectively, it is tempting to hypothesize that a CD14/TLR4 mediated association exists flanked by infection and the advance of acute coronary events. Additional sound designed potential trials are desirable to identify completely the function of genetic polymorphisms in atherogenesis and the progress of cardiovascular events.

## Acknowledgement

None

## Conflict of Interest

The authors hereby declare no conflict of interest.

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