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IL18RAP polymorphisms and its plasma levels in patients with Lumbar disc degeneration

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ABSTRACT

Objectives: Lumbar disc degeneration (LDD) is a common musculoskeletal disorder. Interleukin 18 Receptor Accessory Protein (*IL18RAP*) gene is involved in disc degeneration and inflammatory processes like matrix degeneration. Hence, this study was performed to understand the role of 2 *IL18RAP* (rs1420106 and rs917997) polymorphisms and IL18RAP plasma levels in lumbar disc degeneration (LDD) in Indian population.
Patients and methods: 200 LDD patients and 200 healthy controls were recruited for the study. Genotyping was performed using allelic discrimination assay. IL18RAP levels were measured by ELISA.
Results: rs1420106 polymorphism did not follow Hardy Weinberg equilibrium, so it was not considered for association analysis. There was a significant association among females in CT genotype of rs917997 in LDD ($p = 0.041$). Also, among subjects with no history of alcohol consumption, CT allele was found to be significantly associated and had a protective effect (OR = 0.61). The plasma levels of IL18RAP were also measured. There was no significant difference in IL18RAP levels between patients and controls.
Conclusion: Overall, rs917997 polymorphism did not show any significant difference between patients and controls ($p = 0.77$). However, it showed a protective role in females and patients with no history of alcohol consumption in Indian population and there was no association between polymorphisms and IL18RAP plasma levels.

1. Introduction

Low back pain is the leading cause of disability in India and worldwide and it affects 70–85% of individuals atleast once during their lifetime [1]. Lumbar disc degeneration (LDD) is the main cause of low back pain [2]. LDD is a multifactorial disease with genetic and environmental factors contributing to its etiology [3,4]. Environmental risk factors like smoking and physical labour are proven to cause LDD [5,6]. The genetic etiology of the disease still remains uncertain. Recent research suggests that the factors responsible for disc degeneration may have key genetic components. A strong familial predisposition is reported for disc degeneration and herniation [7]. Two different twin studies have shown that the heritability of LDD exceeds 60% [4,8].

Genes coding for molecular components of the disc and associated biochemical pathways have been explored as candidate genes for LDD. Also, genes involved in inflammation, matrix turnover and degradation are ideal candidates to be analyzed for studying the role of genetics in disc degeneration. Several studies have found significant associations with different pathologic changes of disc degeneration and clinical phenotypes for the polymorphisms in the following genes: aggrecan, collagens (*COL1A1, COL9A1, COL9A2, COL9A3, COL11A1, COL11A2*), vitamin D receptor, matrix metalloproteinases (*MMP2, MMP3, MMP9*), interleukins (*IL1, IL2, IL18R1, IL18RAP*), cyclooxygenase-2 and cartilage intermediate layer protein [9].

IL18RAP gene encodes a protein which is an accessory subunit of the heterodimeric receptor for a pro-inflammatory cytokine interleukin 18 (IL18) [10]. This protein enhances the IL18-binding activity of the IL18 receptor and plays a role in IL18 signalling. Hence, it plays a role in both innate and adaptive immunity [11]. Inflammatory cytokines play an important role in disc degeneration. IL18 mediated activation of T cells and natural killer cells results in the secretion of interferon gamma (IFN-γ), which in turn activates macrophages that secrete cytokines TNF-α and IL1, which cause increased matrix degradation both directly and through activation of proteinases like MMPs [12]. These molecules have been found to be secreted by the cells of degenerated and herniated discs and hence play a role in disc degeneration [13,14].

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There are no available studies on single nucleotide polymorphisms (SNPs) of *IL18RAP* in correlation with its plasma levels. Therefore, in this study, we are exploring two *IL18RAP* polymorphisms (rs1420106 and rs917997) and IL18RAP level in plasma of patients with LDD in Indian population.

2. Materials and methods

2.1. Study population

200 patients with Lumbar Disc Degeneration were recruited from the Department of Neurosurgery, National Institute of Mental Health and Neurosciences, Bangalore, India, for this study during January 2016 to April 2018. Patients with low back ache with sciatica and whose MRI showed lumbar disc degeneration were included into the study and patients with physical damage to the disc and other genetic disorders were excluded from the study. The age and sex- matched healthy controls (n = 200) were randomly selected from general population who were healthy adults without a history of back pain in the recent past. The healthy controls were unrelated to patients but were of the same ethnicity. This study was approved by Institute ethics committee for human studies, NIMHANS, Bangalore, India. Informed consent was obtained from all the participants involved in this study. Five ml of blood samples were collected from patients and control subjects for analysis. The blood samples were processed to obtain plasma and whole blood was used for DNA isolation. The MRI of the patients were analysed as given in Riesenburger et al. and the total MRI score was calculated [37]. The median score was 8 and the patients were divided into 2 groups: ≤8 (low disc degeneration) and > 8 (high disc degeneration).

2.2. Genotyping

Macherey-Nagel (MN) kits were used to extract genomic DNA from blood according to manufacturer's protocol. Nanodrop ND2000c spectrophotometer was used for quantitation and purity checking. DNA with A260/280 1.75–1.85 was used for genotyping. Genotyping of the was performed using Taqman® allelic discrimination assay (Applied Biosystems, Foster City, CA) with a commercially available primer probe set (assay ID C__8906059_10 for rs1420106, C___345197_1 for rs917997). Genotyping was performed in Applied Biosystem 7500 Fast machine.

2.3. ELISA

ILl8RAP levels in the plasma of patients and controls was measured using Human IL-18RB (IL18RAP) ELISA Kit (Cat no: EHIL1RAP, Thermo Scientific, USA) according to manufacturer's instructions. The sensitivity of this assay is 12 pg/mL. Briefly, 100 µL of each standard and sample were added into appropriate wells and incubated for 2.5 h at room temperature with gentle shaking. Solution was discarded and washed 4 times with 1X Wash Buffer. 100 µL of 1X prepared biotinylated antibody was added to each well and incubated for 1 h at room temperature with gentle shaking. Solution was discarded and washed 4 times with 1X Wash Buffer. 100 µL of prepared Streptavidin-HRP solution was added to each well and incubated for 45 min at room temperature with gentle shaking. Solution was discarded and washed 4 times with 1X Wash Buffer. 100 µL of TMB Substrate was added to each well and incubated for 30 min at room temperature in the dark with gentle shaking. Absorbance was measured at 450 nm and 550 nm. 550 nm values were subtracted from 450 nm values to correct for optical imperfections in the microplate.

2.4. Statistical analysis

Statistical software R.3.3.1 was used for analysis of data.

Table 1
Demographic and clinical characteristics of patients with LDD and healthy controls.

Characteristics	Healthy controls (n = 200)	Patients (n = 200)	*p*- value
Mean age (years ± SD)	42.92 ± 18.23	42.94 ± 12.47	0.543
Males n (%)	117 (58.5)	128 (64.0)	0.259
Females n (%)	83 (41.5)	72 (36.0)	
Smoking History n (%)			
Yes	–	48 (24.0)	
No	–	152 (76)	
Alcohol Consumption n (%)			
Yes	–	45 (22.5)	
No	–	155 (77.5)	
MRI Score (n = 136)			
> 8 n (%)	–	66 (48.5)	
≤8 n (%)	–	70 (51.5)	

Continuous variables were expressed as mean ± SD and categorical variables are expressed as percentage values or absolute numbers. $\chi 2$ test was used to find out the difference in genotype and allele frequencies between groups. Association between *IL18RAP* genotypes or alleles and LDD risk were expressed as Odds Ratio with 95% Confidence Interval (CI). Mann-Whitney U test and Kruskal–Wallis test was used to determine the association of the IL18RAP protein levels with disease, genotype and other variables between different groups. The Hardy-Weinberg equilibrium calculation and additive effect of SNP was calculated using the online tool SNPStats, https://www.snpstats.net/start.html. The results were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Clinical and demographical details of study subjects

Demographic and clinical details of patients and controls were given in Table 1. Patients included in the study were in age ranges between 18–80 years. There were 72 females (mean age: 42.67 ± 12.09) and 128 males (mean age: 43.09 ± 12.73) in LDD patients. The healthy control subjects recruited were in the age group 18–80 years. The control group consisted of 83 females (mean age: 43.52 ± 20.38) and 117 males (mean age: 42.70 ± 16.64). There was no significant difference in the mean age and gender among patients and controls.

3.2. IL18RAP polymorphism and risk of LDD

The distribution of *IL18RAP* genotypes and allele frequency was given in Table 2. For rs917997, the distribution of genotype frequencies of controls was in Hardy–Weinberg equilibrium (HWE) (p = 0.16). The rs1420106 SNP did not follow HWE (p < 0.001). Therefore, it was not considered for further analysis. In our study, for rs917997 there was no significant difference in genotypes ($\chi 2 = 2.070$; df = 2; p = 0.354) and allele frequencies ($\chi 2 = 0.045$; df = 2; p = .831) between cases and controls. However, gender stratification of samples reported that the overall genotype distribution of rs917997 polymorphism was differentially distributed in female patients and controls ($\chi 2 = 7.434$; df = 2; p = 0.024). The CT genotype was more prevalent in female controls than in female cases, genotype frequencies being 57.8% and 36.2% respectively.

Logistic regression analysis of the effect of variant indicated that CT genotype was associated with lower risk of LDD in female gender (OR = 0.45; 95%CI = 0.21-0.97; p = 0.041). However, in males the CT genotype was not significantly associated with LDD risk (OR = 1.11; 95%CI = 0.61–2.02; p = 0.734). Also, the CT genotype had a protective effect among patients with no history of alcohol consumption (OR = 0.61; 95%CI = 0.37–1.00; p = 0.05). Also, T allele was not

Table 2
Genotype and allele frequency of rs917997 SNP in LDD patients and controls.

rs917997 Group	Genotype count (%)			*p*- value† (genotype)	Allele frequency		OR (95%CI) *p*- value‖(alleles)
	CC	CT	TT		C	T	
All							
Cases (n = 200)	57(28.5)	90 (45)	53(26.5)	0.354	0.51	0.49	1.04 (0.79-1.38)
Controls (n = 200)	52 (26)	104(52)	44 (22)		0.52	0.48	0.831
Females							
Cases (n = 72)	23 (1.9)	26(36.2)	23(31.9)	0.0243*	0.50	0.50	0.93 (0.59-1.45)
Controls (n = 83)	19(22.9)	48(57.8)	16(19.3)		0.52	0.48	0.839
Males							
Cases (n = 128)	34(26.6)	64(50.0)	30(23.4)	0.9401	0.52	0.48	1.02 (0.72-1.46)
Controls (n = 117)	33(28.2)	56(47.9)	28(23.9)		0.52	0.48	0.971

* Statistically significant.
† test of comparison of genotype distributions (Fisher's exact test).
‖ test of comparison of allele distributions (Fisher's exact test); CI = confidence interval.

Table 3
Logistic regression analysis of association between rs917997 SNP and LDD risk.

Model of inheritance (rs917997)	OR (95%CI)	*p*-value
CC + CT Vs TT	1.29(0.57-1.37)	0.28
CC Vs CT + TT	0.88(0.57-1.37)	0.58
CC Vs TT	1.09(0.63-1.90)	0.736
CC Vs CT	0.79(0.49-1.26)	0.324
Log-Additive model	1.04(0.79-1.37)	0.77

associated with LDD patients with MRI score > 8 (OR = 1.19; 95%CI = 0.80–1.76; p = 0.395) and MRI score ≤ 8 (OR = 1.29; 95%CI = 0.87-0.89; p = 0.201). None of the models of inheritance were significantly associated with LDD risk in our study. The result of logistic regression analyses is shown in Table 3.

3.3. IL18RAP level and risk of LDD

IL18RAP ELISA was performed for 74 patients and 14 controls. The protein levels were obtained for 43 patients and 13 controls. There was no significant difference between mean age and gender between cases and controls (p = 0.365 and p = 0.568 for age and gender, respectively). There was no significant association between IL18RAP plasma levels in cases and control (12.69 Vs 20.03 pg/ml, $p = 0.093$). In LDD cases, IL18RAP plasma levels were compared between different groups (gender, age, smoking history and alcohol consumption, *IL18RAP* polymorphism). No association was found between the plasma levels of IL18RAP and different groups as shown in supplementary Table 1.

4. Discussion

Genetic factors play an important role in the patho-physiology of LDD. Molecular biomarkers are becoming increasingly important as indicators of the presence of a disease, and in evaluating outcome during the treatment. Disc degeneration is a multifactorial process which is influenced by a variety of factors like genetic predisposition, aging, lifestyle conditions and other health factors. Prominent infiltration of inflammatory cells mostly macrophages have been observed in histologic evaluation of herniated disc tissue [15,16]. TNFα, IL-1β, and IL-6 showed a larger presence in surgical specimens of herniated disc tissue when compared with autopsy control tissue specimens and explants cultures, hence, demonstrating a heightened release of IL-6, IL-8, prostaglandin E2 and nitric oxide from herniated disc tissue compared with control tissue [17–19]. Studies have also revealed that IL-12 and IFNγ are majorly expressed in herniated disc fragments compared with bulging discs that remain contained within the disc space by an intact annulus fibrosus [20]. Immune system and inflammation cascades play an important role in disc degeneration [21]. In the interveterbral discs pro-inflammatory cytokines stimulate the production of MMPs and inhibit tissue inhibitor of metalloproteinases, which lead to increased disc degeneration [22,23].

As many genes are involved in disc degeneration, SNPs in those genes play a major role in disease development and progression. Research findings of associations between disc degeneration and gene polymorphisms of matrix macromolecules have confirmed genetic predisposition of LDD [24]. Until now the approach has been via searching for candidate genes, with the main focus being extracellularmatrix genes. Even though there is a lack of association between disc degeneration and polymorphisms of the major collagens in the disc, collagen types I and II [25], mutations of two collagen type IX genes, namely *COL9A2* and *COL9A3*, have been strongly associated with lumbar disc degeneration and sciatica in Finnish population [26,27].

IL18 is an important pro-inflammatory cytokine which is involved in disc degeneration. It is mainly produced by activated macrophages, Kupffer cells and dendritic cells and it upregulates several cytokines like IFN-γ, TNF-α and IL1β [28,29]. The IL18 receptor includes the IL18 receptor accessory protein (IL18RAP) and IL18 receptor 1 (IL18R1) protein [30]. IL18 binds to its receptor IL18R1 protein and triggers the recruitment of IL18RAP and initiates signalling. IL18RAP is essential for IL18 signalling and it forms the signalling chain of this receptor complex and results in the production of several cytokines [31]. As IL18RAP is a key factor for IL18 signalling, polymorphisms in the receptor gene can play a role in LDD.

rs1420106 is an upstream gene variant of *IL18RAP* gene and rs917997 is a downstream gene variant. rs917997 was found to have a strong association with Crohn's disease and ulcerative colitis [32]. It has been found that *IL18RAP* SNP rs917997 was protective against type 1 diabetes [33]. The *IL18RAP* rs917997 C allele was strongly associated with a protective effect in Barrett's esophagus and esophageal adenocarcinoma [34].

IL18RAP polymorphisms (rs917997 and rs1420100) were associated with disc signal intensity in Finnish twin study [35]. Omair et al., studied the role of these SNPs in the Norwegian population and LDD [9]. They did not find any association of rs1420106 and rs917997 SNPs with risk of LDD. Among Indian population there was one study involving *IL18RAP* polymorphism (rs1420100) [36]. They have analysed the role of 58 SNPs in correlation with MRI characteristics. rs1420100 was found to be significantly associated with the development of annular tears. But, the role of rs1420106 and rs917997 SNPs in LDD has not been studied in Indian population. In our study, the genotypic distribution of rs1420106 is as follows, AA-25%, AG-62% and GG-13%. In our study population, minor allele was G with minor allele frequency of 0.44 in patients and 0.41 in controls. Unfortunately, it did not follow HWE, so we did not consider it for further analysis.

The genotypic distribution of rs917997 is as follows, CC-28.5%, CT-45%, TT-26.5%. In our study, minor allele frequency was 0.49 in patients and 0.48 in controls. The genotypes and alleles were not associated with the risk of LDD. In females, CT allele was significantly associated and was found to have a protective effect (OR = 0.45; CI = 0.21-0.97). Also, CT allele was found to have a protective effect in subjects with no history of alcohol consumption (OR = 0.61; CI = 0.37–1.00). The smoking and drinking status of control samples was not available for control subjects.

The MRI analysis was performed as given in Riesenburger et al. and MRI score was calculated [37]. The median score was 8 and the patients were divided into 2 groups: ≤8 (low disc degeneration) and > 8 (high disc degeneration). rs917997 SNP was not associated with MRI score. We also studied the expression levels of *IL18RAP* protein in plasma samples. We did not find any association with LDD. There was no correlation between plasma levels of *IL18RAP* and its genotypes or alleles.

Various SNPs are being used in clinics/pharmacogenomics to predict the risk and prognosis of various disease conditions. As LDD is a complex disease having various risk factors like environment and genetics, genetic factor alone cannot be used at this level for the prediction of risk of LDD. Much larger studies are required to prove the association of IL18RAP SNP with LDD.

Our study has some limitations. rs1420106 polymorphism did not follow Hardy Weinberg equilibrium, so this SNP could not be used for association analysis. Indian population is heterogeneous in nature and thus this study may not have all the characteristics of an Indian population. The data about history of smoking and alcoholism in control group was not available for the study.

5. Conclusion

IL18RAP gene polymorphisms can play a key role in LDD. CT genotype of rs917997 SNP had a protective effect in females and patients with no history of alcohol consumption. This study is a preliminary study and further studies involving a larger cohort of population may give a better idea. The mRNA and protein expression study in the degenerative discs can help us understand the role of *IL18RAP* more clearly.

Declaration of interests

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.clineuro.2019.105374.

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