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RESEARCH ARTICLE
НАУЧНАЯ СТАТЬЯ

Association of BTNL2 gene single nucleotide polymorphism with knee osteoarthritis

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Abstract. *Relevance*. Osteoarthritis (OA) is one of the chronic debilitating condition mostly seen in the aged population. The etiology behind the OA is multifactorial and the exact cause of the disease often remains uncertain. Apart from the conventional risk factors, there are the speculations of role of genetics playing a pivotal role in the causation of OA. The available literature showed BTNL2 gene polymorphism association with risk of Osteoarthritis whether the same relation is present in north Indian population needs to be elucidated. *Objective*. To find the association between single nucleotide polymorphism (SNP) (rs10947262) in BTNL2 gene and the susceptibility in knee Osteoarthritis (OA) subjects from northern Indian population. *Materials and Methods*. Blood samples of 100 patients of knee osteoarthritis and 100 healthy subjects were collected after institutional ethical clearance and participants consent. The BTNL2 gene fragment was amplified using Amplification Refractory Mutation System (ARMS-PCR) with predesigned primers after DNA extraction. The corresponding product bands were identified on the gel electrophoresis for 200 samples and the results were statistically analyzed. *Results and Discussion*. The genotypic distribution of the SNP followed Hardy-Weinberg Equilibrium. The genotype frequency analysis of the polymorphism was statistically significant ($\chi 2=7.788$; $P=0.005$) with Odd's Ratio of CT+TT/CC: OR=2.303; P=0.008 revealing association of BTNL2 polymorphism with risk of Knee Osteoarthritis. *Conclusion*. The SNP (rs10947262) in the BTNL2 gene region is associated with risk of knee osteoarthritis.

Key words. Osteoarthritis, BTNL2 polymorphism, Cytokine, Knee

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Introduction

Geriatric population constitute a significant proportion of world total population. Geriatric age is often accompanied by senile disorders with restricted movement among the age group. Musculoskeletal deformities are often seen in this age along with hypertension, Diabetes Mellitus or other lifestyle disorders. Osteoarthritis (OA) is a age related joint disease causing significant disability in aged population of developed as well as developing countries standing as fourth leading cause of years lived with disability [1]. It is complex disease of multifactorial etiology including genetic and environmental factors [2]. It is basically inability to maintain the balance between cartilage breakdown and regeneration [3]. OA is considered as a non-inflammatory arthropathy however, inflammatory factors have also seen involved in the pathogenesis of OA [4].

Various risk factors for Osteoarthritis have been identified including Female gender, obesity, bony deformities and Joint injuries [5]. The propensity of this disease running in families is also observed and are associated with human leukocyte antigen (HLA) type I and II [6]. It is also seen that low-grade inflammation mediated by cytokines may occur in the OA [3]. While various studies tried to establish the genetic risk of having osteoarthritis among different ethnicities, a genome-wide association scan in Japanese population identified role of BTNL2 gene polymorphism strongly associated with knee OA [7]. The HLA class I and class II genes are responsible for the development of osteoarthritis apart from their essential role in self/non-self-immune recognition [8].

Some Asians have low risk of developing Osteoarthritis [9]. However, its prevalence in India was documented about 22 to 39 percent in one study [10]. Most of the Asian countries are developing one and many people are still devoid of essential nutrition. They have high chance of getting OA via trauma or joint overuse or from genetic influence along with existing osteoporosis. Until now, 90 genetic variations are found to be associated with the risk of osteoarthritis [11]. These variations are heritable determinants for the causation of the disease and need to be studied further to understand the risk of osteoarthritis in the Indian Population.

Materials and methods

The study was approved by the ethical committee of the Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi. All patients gave informed consent to participate in the study according to the Helsinki Declaration of the World Medical Association (WMA Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects, 2013) and personal data processing. The basic parameters like age, weight, height, gender of each patient were noted. Cases included 100 adults of either gender above 45 years of age diagnosed as Osteoarthritis from Orthopedics Department while 100 apparently healthy adults of both the sexes, above 45 years of age without any history of joint pain or any other skeletal disorder were enrolled in the study. Diagnosis of Osteoarthritis of knee was done as per the criteria laid by American College of Rheumatology followed by taking radiographs of affected knee joint for radiological scoring by Kellgren and Lawrence scores (KL scores). A KL score of 2 or more was classified as OA. Diagnostic criteria of American college of Rheumatology includes knee pain with any 3 of the following criteria [12, 13]:

1. Over 50 years of age;
2. < 30 minutes of morning stiffness;
3. Crepitus on active motion;
4. Bony tenderness;
5. Bony enlargement;
6. No palpable warmth of synovium.

Participants having underlying comorbidity like

Rheumatoid arthritis, Gout, Congenital lower limb deformity or history of severe trauma to the affected joint were excluded from the study.

Under strict aseptic conditions 2 ml of venous blood was collected in EDTA vacutainer and stored at -70C.The DNA isolation was done by «QIAGEN DNA extraction Kit» follow by amplification by polymerase chain reaction (PCR). The loci for BTNL2 gene SNP (rs10947262) was identified by using Amplification Refractory Mutation System (ARMS-PCR). The primers used for amplification were:

- ancestral gene sense strand(A): 5'-GTCACCTACCAGCTATGTGAGTC-3';
- polymorphic gene Sense strand(P): 5'- GTCACCTACCAGCTATGTGAGTT-3';
- common for antisense strand (R): 5'-CAAACCAGTGTCCTTAATCCAGC-3'.

Each 20µl of PCR mixture consisted of 200—300 ng of Template DNA, 0.3µl of sense and anti-sense primers in respective ancestral and polymorphic PCR tubes, 20 mM of MgCl2 (present in PCR Buffer), 0.4µl of dNTPs (a mixture of 10 mM each of dATP, dCTP, dGTP, dTTP), 0.9µl of 10X PCR buffer, 0.3µl of Taq DNA polymerase, 17.7µl of ddH2O (DNase/RNase-free) was used. The reaction mixture was subjected to denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, then a final extension at 72 °C for 5 min. 2 % Agarose gel electrophoresis was performed to view the PCR product of 421bp size.

Statistical Analysis. Quantitative variables were expressed as Mean ± SD. The differences in the general characteristics between the cases and the control group were evaluated via Student t-test. The difference in genotype distribution and sex ratio between the groups was evaluated by Pearson's $\chi 2$ test. Genotypic and allelic frequencies were calculated by direct counting. Hardy-Weinberg equilibrium (HWE) test was applied to confirm the independent segregation of the alleles. Odd's Ratio (OR) was used to assess the association of SNP and osteoarthritis of the knee. A two-sided $P<0.05$ was considered statistically significant.

Results and discussion

A comparison of the baseline characteristics are mentioned in Table 1.

Table 1

Baseline characteristics of participants

Parameters	Cases (N=100)	Controls (N=100)	$\chi 2$/ t- value	p-value
Age, years	59.56±11.5	58.46±10.6	0.721	0.471
Female Gender, %	63	56	1.02	0.312
Weight, kg	78.85±11.5	76.76±19.3	0.930	0.353

There was no significant difference between the mean age (years) 59.56±11.5 vs. 58.46±10.6, weight (kg) 78.85±11.5 vs. 76.76±19.3 as well as the sex ratio among the case and control groups ($P>0.05$).

The amplification of genomic DNA yielded PCR products of 421 base pairs (bp) on Agarose gel electrophoresis (Figure 1).

The PCR products were detected by comparing it with a DNA ladder which was ran along with the PCR products, where CC (homozygous Ancestral), CT (heterozygous polymorphic), and TT (homozygous polymorphic). The SNP was within the Hardy-Weinberg equilibrium with $P=0.22$ (Table 2).

In this study, the SNP (rs10947262) in the Butyrophilin like 2 (BTNL2) gene region was evaluated using chi-square test. The genotype CT+ TT cumulatively was statistically significant ($\chi 2 =7.788$; $P=0.005$) (Table 3).

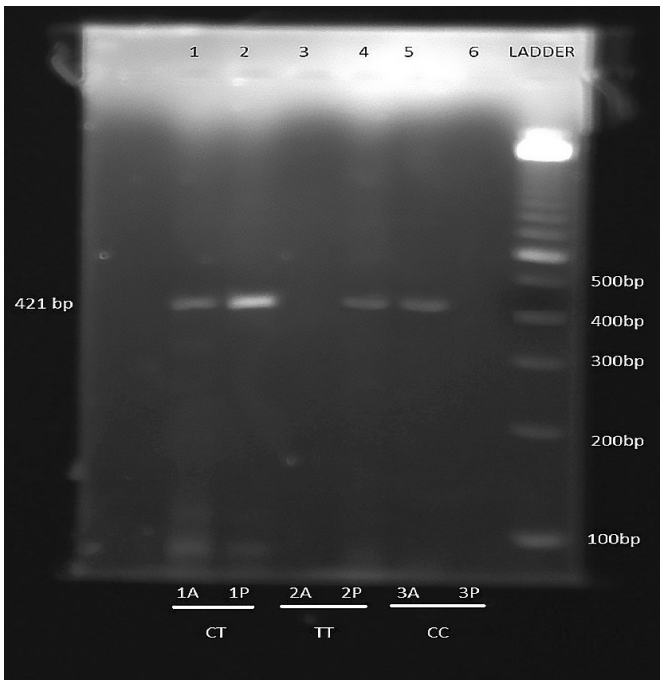

Fig. 1. Agarose gel picture of BTNL 2 gene SNP rs10947262. A- ancestral; P-polymorphic. Lane 1,2-Heterozygous (CT), Lane 3,4-Homozygous polymorphic (TT), Lane 5,6-Homozygous ancestral. bp=base pairs

When Odd's Ratio (OR) was applied to find the association between the polymorphism and risk of Osteoarthritis, the OR values for SNP were statistically significant (CT+TT/ CC: OR=2.303; P=0.008) indicating the BTNL2 polymorphism is associated with risk of osteoarthritis (Table 3).

While various hypothesis in the causation of Osteoarthritis have been postulated, significant association of abnormal joint anatomy causing increased focal stress or an acute injury or chronic stress in obese individuals or a combined effect have been seen to cause the OA. The disease has association with the obese individuals bringing stress to joints and imbalance between cartilage regeneration and degradation however, persons well within normal BMI also have documented incidence of OA. Hence, the genetic variability needs to be known. Present treatment options include supportive management, Physiotherapy and joint replacement therapy and exploring the genetic susceptibility targeted management could help to prevent the disease itself or at least halt the progression of the disease as some osteoarthritis patients invariably will require joint replacement and joint replacement depends upon the socio-economic status of the patients [14].

Table 2

Hardy Weinberg Equilibrium of SNP of rs10947262

Alleles	Frequency/	χ2	P-value/
CC	73	3.027	0.22
CT	105		
TT	22		

Table 3

Genotype frequency analysis of SNP & its association with risk of OA

Alleles	Cases (N=100)	Controls (N=100)	χ2	P-value	ODD'S Ratio	P-value
CC	27	46	–	–	1 (ref)	–
CT	62	43	8.38	0.004*	2.456	0.006*
TT	11	11	1.192	0.274	1.704	0.324
CC/(CT+TT)	73	54	7.788	0.005*	2.303	0.008*

*p<0.05

A large-scale study in European descent population involving more than 5000 cases of OA and more than 36000 controls revealed two SNPs rs10947262 and rs7775228 variants has no association with increased risk of OA [15]. This finding is contrary to our study findings where rs10947262, CT and TT genotype were associated with increased risk of osteoarthritis. Another Japanese study associated with two SNPs of BTNL2 region including our studied reference sequence showed significant association of these genotype with risk of OA [5]. The same association was also echoed in a Chinese study, where four SNP from BTNL2 gene region were evaluated rs41521946, rs28362677, rs28362678, rs28362675 and found significantly associated with knee osteoarthritis, similar to our study however, we have evaluated rs10947262 of the same gene [16]. In another study by Shi D et al. SNP rs10947262 was evaluated in Han Chinese and Australian Caucasian population [17]. This study showed no association of risk of osteoarthritis with SNP in Han Chinese population while same study revealed significant risk with Caucasian population, Similar to genome wide study conducted in European descent population.

Various immune cells including T cells, B cells, and macrophages are seen to be involved in the pathogenesis of OA same like Rheumatoid arthritis however, the role of cytokine directed therapy is seen in Rheumatoid arthritis and very few studies have echoed anti cytokine therapy in Osteoarthritis [18, 19]. BTNL2 gene is responsible for inhibition of T cell activation, which have role in osteoarthritis and its polymorphism may lead to unopposed T cell activation and joint damage [20]. Apart from this, when HLA II gets attached to the Antigen (wear & tear cartilage fragments), it activates the macrophages (APCs) which in turn releases IL-1, IL-12, TNF-α, vascular endothelial GF, chemokine (CXCL12) [21]. It also releases osteoinducive factors (Bone Morphogenetic Proteins) which helps in osteophytes formation, IL-12 which is a inducer of Th1 cells that releases IL-2, IL-10, IFN-γ, where IL-2 promotes Th1 cell further differentiation [22, 23]. IFN-γ induces macrophages & HLA-II expression & inhibit collagen type II synthesis that lead to decrease in cartilage formation [24]. CXCL12 induces migration and retention of monocytes into synovial membrane. These then over all activates T cells that produce collagenase in the synovial membrane that lead to destruction of cartilage leading to OA [25, 26].

Hence further and extensive research in this field is required as the current treatments for OA are limited and insufficient to prevent the occurrence and progression of the disease. Genetic studies of OA patients can throw insight into the molecular mechanisms that lead to the progression of the diseases, including joint damage and pain and can provide new approach towards treatment as well as prevention of the disease.

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Ассоциация однонуклеотидного полиморфизма гена BTNL2 с остеоартрозом коленного сустава

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Аннотация. *Актуальность*. Остеоартрит (ОА) является одним из хронических заболеваний, чаще всего наблюдаемым у пожилых людей. Этиология остеоартрита многофакторна, и точная причина заболевания часто остается неопределенной. Помимо общепринятых факторов риска существуют предположения о том, что генетика играет ключевую роль в возникновении ОА. В литературе показана связь полиморфизма гена BTNL2 с риском остеоартрита, необходимо выяснить, присутствует ли такая же связь в популяции северной Индии. *Цель*. Определить, существует ли связь между однонуклеотидным полиморфизмом (SNP) (rs10947262) в гене BTNL2 и восприимчивостью к остеоартриту (ОА) коленного сустава из населения Северной Индии. *Материалы и методы*. Образцы крови 100 пациентов с остеоартрозом коленного сустава и 100 здоровых доноров были собраны после разрешения этического комитета Медицинского колледжа Вардхмана Махавира и согласия участников. Фрагмент гена BTNL2 амплифицировали с использованием системы устойчивых к амплификации мутаций (ARMS-PCR) с предварительно созданными праймерами после экстракции ДНК. Соответствующие полосы продукта были идентифицированы с помощью гель-электрофореза для 200 образцов, а результаты были проанализированы статистически. *Результаты и обсуждение*. Генотипическое распределение SNP соответствовало равновесию Харди-Вайнберга. Анализ частоты генотипов полиморфизма был статистически значимым ($\chi 2 = 7{,}788$; $P = 0{,}005$) с отношением шансов CT + TT / CC: OR = 2,303; P = 0,008 указывает на связь полиморфизма BTNL2 с риском остеоартрита коленного сустава. *Выводы*. SNP (rs10947262) в области гена BTNL2 связан с риском остеоартрита коленного сустава.

Ключевые слова: остеоартрит, полиморфизм BTNL2, цитокин, колено

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