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Association of Polymorphism in Growth and Differentiation Factor 5 Gene with Osteoarthritis Knee

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ABSTRACT

The present study investigated to identify the association of polymorphism in GDF-5 gene with osteoarthritis in North Indian population. In a case-control study, 300 cases with knee osteoarthritis and an equal number of age and gender matched healthy controls were included. Cases were diagnosed using the ACR Guidelines of Knee Osteoarthritis (KOA). Clinical symptoms were assessed with the knee specific WOMAC index and VAS for knee pain. The severity of disease was determined by radiological KL grades (Kellgren Lawren). The variant genotype of GDF-5 was found to be present at significantly higher frequency in cases than in controls, resulting in about 1.79 fold increase in risk to OA. Genotype distributions of the GDF-5 also showed significant association of variant allele with clinical score of OA patients. An association between the+104T/C GDF5 polymorphism with knee OA and clinical symptom of OA in Indian population further demonstrate a strong genetic influence of this SNP in KOA.

Keywords: Polymorphism in GDF-5, Clinical Symptoms, Knee Osteoarthritis, Indian Population

1. INTRODUCTION

Osteoarthritis (OA), characterized by gradual loss of articular cartilage in the joint, is a leading cause of disability among the elderly people (Altman, 2011; Ding *et al*., 2008). Though the etiology and pathogenesis of OA is largely unknown, OA is a chronic degenerative condition of mobile joints, primarily a non-inflammatory disorder characterized by an imbalance between the synthesis and degradation of articular cartilage leading to classic pathological change of wearing away and destruction of cartilage (Brandt *et al*., 2009). It has been observed that about 80% of population has radiographic evidence of OA by the age of 65 years, although only about 60% of these were symptomatic (Green, 2001). Epidemiological profile of OA in India is not very clear but it is estimated that more than 30-40% of our population suffer from osteoarthritis beyond the age of 50 years (Sharma *et al*., 2007). Hip and knee OA represent a huge healthcare burden to society and a personal burden to individuals affected by the disease, in addition to being the main cause of the increasing need for joint replacements (Valdes and Spector, 2011).

Though the mechanism underlying the development OA is yet to be understood, several biochemichal pathways have been found to be associated with the development of OA (Tchetina, 2011; Valdes and Spector, 2009). An early OA articular cartilage degeneration is associated with increased collagenase cleavage of type II collagen (Buckwalter and Mankin, 1998; Tchetina *et al*., 2006). Collagenases (MMP-1,

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MMP-14 MT1-MMP), aggrecanase ADAMTS-5, cytokines chondrocyte terminal differentiation-related genes and caspase 3 are reported to be upregulated in the vicinity of the lesion (Hardingham *et al*., 1992; Watanabe *et al*., 1998; Tetlow *et al*., 2001; Aigner and Stove, 2003).The most severely damaged rodent knee OA articular cartilage has shown to be significantly associated with reduced expression of proliferation-related growth factors and their signaling molecules such as PTHrP, TGFβ3 and Smad-2P, TGFβ1 and its receptor II (Gomez-Barrena *et al*., 2004; Davidson *et al*., 2006).

Genetic polymorphism in MMP-1 (-1,607 1G/2G), MMP-2 (-1,306 C/T) and MMP-9 (-1,562 C/T), associated with increased transcriptional activity has shown to be involved in inhancing the susceptibility to knee osteoarthritis (Barlas *et al*., 2009). Likewise, allelic variants of ASPN, CALM-1, COL2A1, COMP and ESR-α, GDF-5, VDR, ADAM12 and BMP2 have been reported to be associated with knee OA. (Bergink *et al*., 2003; Mabuchi *et al*., 2003; Jin *et al*., 2004; Kizawa *et al*., 2005; Valdes and Spector, 2009). Growth and differentiation factor 5 (GDF-5), also known as cartilage derived morphogenetic protein of Transforming Growth Factor-b (TGF-b) superfamily, plays an important role in the development, maintenance and repair of bone and cartilage. It has been shown that reduced transcriptional activity leads to decrease in cartilage synthesis (Francis-West *et al*., 1999; Hatakeyama *et al*., 2004; Southam *et al*., 2007) and to compensate for the cartilage space, the bone beneath thickens and spreads out form knobbly outgrowths (osteophytes).

As polymorphism in GDF-5 may lead to decreased cartilage synthesis, attempts were made in the present study to explore the association of genetic polymorphism in GDF5 with OA in North Indian population. Attempts were also made to correlate variant T allele of GDF-5 with clinical profile of OA by associates GDF-5 polymorphism with one of the most widely utilized self-report measures of lower extremity symptoms and function, the Western Ontario Mac University (WOMAC) index and Visual Analogue Scale (VAS) for knee pain.

2. MATERIALS AND METHODS

2.1. Study Subjects

This Case control study consisted of men and women ≥40 years that fulfilled American College of Rheumatology (ACR) (Sanghi *et al*., 2011) clinical and radiographic criteria for KOA. Cases (300) and equal no of controls were recruited from the outpatient clinic of the Department of Orthopaedic Surgery of C. S. M. Medical University. The patients and controls were matched for age and sex. These patients were profiled for demographic, clinical, radiological and biochemical features. Age and sex were self reported. Patients were weighed with a calibrated balance beam scale to the nearest 0.1 kg in minimum possible clothing and standing height was measured (shoes and shocks) with a Stadiometer in centimeters (cm). Body Mass Index (BMI) was recorded by Quetelet index. Among the cases104 were men and 196 were women and age range from 40-72, mean age 54.49±9.24 and 53.75±8.47 years respectively. In the controls 123 were male with mean age 54.50±7.61 years and 177 female with mean age 55.65±8.59 years. The protocol for research work was approved by the human ethics committee of C.S.M. Medical University, Lucknow. The protocol conforms to the provisions of the declaration of Helsinki in 1995. Informed consent was obtained from the study subjects for inclusion in the study and before the collection of blood samples and it was also ensured that subject anonymity was preserved. The control and cases were asked to fill up the detailed questionnaire regarding their occupation, socioeconomic status, medical history, life style habits. The Kellgren-Lawrence grade represents disease severity, as reflected on radiographs, Radiographic findings of OA were classified into mild (K-L grade 2), moderate (K-L grade 3) severe (K-L grade 4).Number of participants in KL grades 2, 3 and 4 were 71, 174 and 55, respectively. Average BMI was 25.52±3.58. Symptoms related to KOA were assessed with the knee-specific WOMAC index (Bellamy, 1989), which assess pain (five items), stiffness (two items), function (17 items) and interpretation response in terms of a 5-point scale (0, none; 1, slight; 2, moderate; 3, severe; 4, extreme). Knee pain was also assessed using the VAS, where higher scores indicate worse status.

2.2. DNA Isolation and Genotype Analysis

Approximately 1 ml of blood was collected into citrate containing tubes from all the subjects. DNA was isolated from whole blood with the Flexi Gene DNA kit (Qiagen, CA) following the manufacturer's protocol. Isolated DNA was subsequently used for genotyping studies.

2.3. Detection of GDF-5 Gene Polymorphisms

The method of (Southam *et al*., 2007) was followed for determining the GDF-5 gene BSiE1 (T/C; rs143383) polymorphism, In brief, the reaction mixture in 25 µL contained 10X buffer, 1.5mM $MgCl_2$, dNTPs (10mM)

0.2 mM , 0.5 µL of 10 pmol of each primer, 1.5U of Taq polymerase (MBI Fermentas, Germany), 100 ng of genomic DNA and sterile MilliQ water. Amplification was performed on GeneAmp PCR system 9700 (Applied Bio System) using the following protocol: 94°C for 5 min for initial denaturation followed by 35 cycles of 94°C for 60 s, annealing at 51°C for 45 s, extension at 72°C for 1min and final elongation step of 72°C for 10 min. PCR reaction resulted in a 197 bp product. PCR products (10 µL) were digested with 10U of BSiE1 restriction enzyme (MBI Fermentas, Germany) to identify the presence of polymorphic sites in GDF-5 gene. Digestion of 197 bp PCR product of GDF-5 gene with BSiE1 restriction enzyme into two fragments of 106bp and 91bp bp indicates the presence of CC genotype of GDF-5 gene BSiE1 polymorphism. The presence of fragments of three sizes (197, 106 and 91 bp) was indicative of the CT genotype while the undigested 197 bp PCR fragment was indicative of TT genotype of BSiE1 polymorphism of GDF-5 gene.

2.4. Statistical Analysis

Genotype or allele frequencies of GDF-5 gene of BSiE1 polymorphism among cases and controls were determined for Hardy-Weinberg Equilibrium (HWE) using standard chi square **Table 2** statistics. Using binary logistic regression models, we determined the relationship of BSiE1 polymorphism of GDF-5 gene with risk of. All statistical analysis was performed with the SPSS software package (version 16.0 for windows; SPSS Chicago, IL). The power of the present study was found to be >80% as analyzed by power genetic association analysis software (http://dceg.cancer.gov/bb/tools/pga) at the level of significance = 0.05 with sample size of 300 controls, 300 patients.

3. RESULTS

The main characteristic of study population age, sex, BMI and clinicoradiological severity are summarized in **Table 1**. The genotype distribution of the polymorphic GDF-5 in the 5-UTR (BSiE1, C/T) in controls and cases is summarized in. The genotypes of GDF-5 in controls were found to be in Hardy-Weinberg Equilibrium (HWE). As evident from the **Table 2** the frequency of TT genotype was higher in cases (41.33%) when compared to the controls (28.00%). This increase in the frequency of TT genotypes significantly increased the risk {OR: 1.79, (1.14-2.89), p value = 0.016)} for KOA in cases when compared the controls. When the cases were stratified on the basis of gender, the frequency of TT genotype was significantly increased in cases (43.26%) in men when compared to the controls (30.81%) significantly increase the risk {OR: 2.43, (1.01-5.83), 0.046} to OA in male cases. In females, the frequency TT genotype was also higher in cases (40.30%) when compared to the controls (26.55%), though the increase in risk {OR: 1.63, (0.93-3.02)} was found to be relatively less when compared to the risk observed in male cases. Percentage of risk allele T was higher in cases 63% when compared with controls 54.66%. The frequency of variant allele T was also found to be increased in both male and female cases (66.82 in male and 60.90% in female) when compared with controls (56.91% in male and 53.10% in female).

Polymorphism in GDF-5 was also associated with clinical symptoms of OA such as the knee specific WOMAC index and VAS for knee pain. OA patients who are functionally or symptomatically poor (poor index) are defined as those with a WOMAC index score and VAS more than 33 and 6 while those with WOMAC index score and VAS less than or equal to 33 and 6 are classified as functionally or symptomatically good (good index). Cases with variant genotype of the GDF-5 had WOMAC index and VAS score of 43.31 and 40.45% respectively. In contrast cases with CC genotype of the GDF-5 had WOMAC index and VAS score of 14.64 and 15.45% respectively (**Table 3**). A summary of association of GDF-5 polymorphisms with radiological features is depicted in **Table 4**.The polymorphism was significantly associated with the individual radiological features of OA knee. There was a significant difference between patients with osteophyte and without osteophyte in right knee ($\chi^2 = 9.04$; P = 0.011) but not between patients with osteophyte in left knee. Likewise juxtraarticular ostopenia was significantly associated in right knee ($\chi^2 = 6.93$; P = 0.031) but not in left knee (**Table 4**). Articular incongruity was significantly associated with both knees. However, no significant association was found with other radiological score.

Table 1. Characteristics of the study population

	Cases (300)	Controls (300)
Age (mean ± SD, years)	54.01±8.74	55.18±8.21
Male age (mean ± SD, years)	54.49±9.24	54.50±7.61
Female age (mean ± SD, years)	53.75±8.47	55.65±8.59
BMI (mean ± SD, kg/m^2)	25.51±3.58	23.77±2.47
Females (n %)	196 (65.33)	177 (59.00)
KL grade 2/3/4	71/174/55	---
VAS (mean ± SD)	6.04±1.07	---
Total womac (mean ± SD)	35.51±9.16	---

Table 2. Genotype association between SNP in GDF-5 gene and Knee Osteoarthritis (KOA)

	All Subjects			Women			Men		
	Control (30)	Case (300)	O R, (95% CI), p value	Control (177)	Case (196)	O R, (95% CI), p value	Control (123)	Case (104)	O R, (95% CI), p value
Rs 143383									
CC	56(18.66)	46 (15.33)	1.00, (Reference)	36(20.33)	36(18.36)	1.00, (Reference)	20(16.26)	10(9.61)	1.00, (Reference)
CT	160(53.33)	130(43.33)	1.00, (0.62 -1.55), 1.000	94(53.10)	81(41.32)	0.86, (0.49-1.49), 0.595	66(53.65)	49(47.11)	1.48, (0.63 -3.45), 0.359
TT	84(28)	124(41.33)	1.79, (1.14 - 2.89), 0.016*	47(26.55)	79(40.30)	1.63, (0.93-3.02), 0.083	37(30.81)	45(43.26)	2.43, (1.01 -5.83), 0.046*
C	272(45.33)	222(37)	1.00, (Reference)	166(46.89)	153(39.03)	1.00, (Reference)	106(43.68)	69(33.17)	1.00, (Reference)
T	328(54.66)	378(63)	1.41, (1.12 -1.78), 0.003*	188(53.10)	239(60.96)	1.38, (1.03-1.84), 0.030*	140(56.91)	139(66.82)	1.53, (1.04 -2.24), 0.030*

* Significant P value

Table 3. Association of genotype with clinical score

	GDF5 (genotype distribution)		
Clinical score	CC (%)	CT (%)	TT (%)
VAS ≤6	34(15.45) $\chi2 = 9.30$	97(44.09) P = 0.010*	89(40.45)
VAS >6	12(15) $\chi2 = 7.29$	33(41.25) P = 0.026*	35(43.75)
Womac ≤33	23(14.64) $\chi2 = 11.0$	66(42.03) P = 0.004*	68 (43.31)
Womac >33	23(16.08) $\chi2 = 5.59$	64(44.75) P = 0.061	56(39.16)

* Significant P value

Table 4. Association of genotype with individual radiological score

	Genotype Distribution (GDF-5)					
	Left			Right		
Radiological score	CC	CT	TT	CC	CT	TT
Joint Space Narrowing (JSN)						
Absent	17	39.00000	26.000	20	37.00000	36.000
Present	29	91.00000	98.000	26	93.00000	88.000
		$\chi2 = 5.13$	P = 0.077		$\chi2 = 3.96$	P = 0.138
Osteophyte						
Absent	0	6.00000	2.000	4	6.00000	0.000
Present	46	124.00000	122.000	42	124.00000	124.000
		$\chi2 = 3.69$	P = 0.158		$\chi2 = 9.04$	P = 0.011*
Articular incongruity						
Absent	14	26.00000	11.000	15	23.00000	15.000
Present	32	104.00000	113.000	31	107.00000	109.000
		$\chi2 = 12.52$	P = 0.002*		$\chi2 = 9.705$	P = 0.008*
Subchondral cyst						
Absent	36	94.00000	79.000	33	94.00000	82.000
Present	10	36.00000	45.000	13	36.00000	42.000
		$\chi2 = 4.11$	P = 0.128		$\chi2 = 1.257$	P = 0.530
Juxta articular osteopenia						
Absent	22	48.00000	51.000	24	40.00000	49.000
Present	24	82.00000	73.000	22	90.00000	75.000
		$\chi2 = 1.73$	P = 0.420		$\chi2 = 6.93$	P = 0.031*
Loose bodies						
Absent	43	126.00000	113.000	43	123.00000	115.000
Present	3	4.00000	11.000	3	7.00000	9.000
		$\chi2 = 3.80$	P = 0.149		$\chi2 = 0.37$	P = 0.827
Extra articular calcification						
Absent	39	109.00000	101.000	40	110.00000	103.000
Present	7	21.00000	23.000	6	20.00000	21.000
		$\chi2 = 0.380$	P = 0.827		$\chi2 = 0.398$	P = 0.819
Subchondral sclerosis						
Absent	40	100.00000	107.000	40	106.00000	110.000
Present	6	30.00000	17.000	6	24.00000	14.000
		$\chi2 = 4.62$	P = 0.099		$\chi2 = 2.72$	P = 0.256

* Significant P value

4. DISCUSSION

Consistent with the previous reports in Caucasian and Orientals (Miyamoto *et al*., 2007; Southam *et al*., 2007), the present study has demonstrated the association between polymorphisms of GDF5 and risk of knee OA in North Indian population and supports the hypothesis that polymorphisms in GDF5 may be involved in the pathogenesis of this disease (Miyamoto *et al*., 2007). Our data have shown that polymorphism exist in the GDf-5 (BsiE1) gene in North Indian population. The frequency of T allele in our population (55%) was similar to that reported in Caucasians (59-63 %) (Rouault *et al*., 2010; Southam *et al*., 2007; Tsezou *et al*., 2008; Valdes and Spector, 2009) the oriental population carry relatively higher frequency (70-74%) of variant T allele (Shin *et al*., 2012; Cao *et al*., 2010; Dai *et al*., 2008; Miyamoto *et al*., 2007).

Our data have shown significant increase in the frequency of T allele of Gdf-5 (BsiE1) gene in KOA when compared with controls. Significant association of polymorphism in GDf-5 (BsiE1) gene has been reported in Oriental and Caucasians population (Southam *et al*., 2007; Valdes and Spector, 2009). Likewise Oriental (Japanese and Chinese) population also showed significant association of polymorphism in GDF-5 with knee OA (Miyamoto *et al*., 2007). However, inconsistent results have also been reported in those populations (Tsezou *et al*., 2008; Cao *et al*., 2010). This has been partly attributed to the smaller sample size and ethinic variations among them. Stratification of subjects on bases of sex further showed significant association of variant T allele with KOA in males and females. Similar association of variant T alleles with KOA in males and females has been demonstrated in Caucasians and Koreans (Cao *et al*., 2010; Tsezou *et al*., 2008; Southam *et al*., 2007).

GDF5 has been found to be expressed in regions of future joints during early development and GDF5 mutations have been associated with abnormal joint development (Francis-West *et al*., 1999). GDF-5 acts at two stages of skeletal development and by two distinct mechanisms. First, GDF-5 promotes the initial stages of chondrogenesis by promoting cell adhesion, which is consistent with the expression of Gdf-5 in the cartilage condensation. Second, GDF-5 can increase the size of the skeletal elements by increasing proliferation within the epiphyseal cartilage adjacent to its expression within the joint interzone (Francis-West *et al*., 1999; Buxton *et al*., 2001). Significant association of polymorphism in GDF-5 with KOA could be related to role of variant T allele to influence transcription of the GDF5 gene with the susceptible allele showing reduced transcriptional activity (Miyamoto *et al*., 2007). The reduced activity of GDF5 may inhibit the process of early cartilage differentiation (Hatakeyama *et al*., 2004). Genetic variations in the GDF5 locus have been reported to be involved in the pathogenesis of rheumatoid arthritis and to influence human height, hip axis length and fracture risk in the elderly (Rouault *et al*., 2010; Vaes *et al*., 2009). Furthermore, association of variant T allele with other musculoskeletal phenotypes, including variation in Achilles tendon pathology, fracture risk and congenital dysplasia of the hip, has also been reported (Posthumus *et al*., 2010; Sanna *et al*., 2008).

Further evidence for the involvement of GDF-5 polymorphism with KOA was provided by our data indicating association of variant (T) allele with clinical symptom of KOA. OA knee was found to be associated with both good and poor index of both VAS and WOMAC. Further, studies are however needed with a larger sample size to identify the possible role of this SNP in GDF-5 with progression of the symptom of KOA with poor and good indices of VAS and WOMAC. A summary of association of variant T allele of GDF-5 with Individual Radiological Features (IRF) revealed that though significant association was not found with all IRF studied; there was a significant difference between patients with and without osteophyte in individuals with right knee OA though no association was found in patients with osteophyte in left knee. Likewise, Juxtraarticular ostopenia was also significantly associated in right knee but not in left knee. Articular incongruity was however significantly associated in both knees, left and right knee suggesting that this polymorphism in GDF-5 is associated with radiological KOA. Sanghi *et al*. (2011) earlier reported that articular incongruity is a better representative of pain and stiffness and juxtra-articular osteopenia and physical disability and clinical severity in OA knee (Sanghi *et al*., 2011).

5. CONCLUSION

This study suggests the association between GDF5 polymorphisms and risk of knee OA in North Indian population and therefore supports the involvement of polymorphism in this gene in the pathogenesis of knee OA. The data suggest that Identification of genetic factors should enable better understanding of the pathogenesis of this complex disease and avoid late diagnosis and delayed treatment.

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