Exploration of Genetic Association Studies with Collagen Variants in Clinically Diagnosed Primary Knee Osteoarthritis in South Indian Population: A Non-replication Study

Krishna Subramanyam[1#], Subhadra Poornima[2#], Imran Ali Khan[3] and Qurratulain Hasan[2*]

[1]*Department of Orthopedics, Kamineni Hospitals, Hyderabad, India*
[2]*Department of Genetics and Molecular Medicine, Kamineni Hospitals, Hyderabad, India*
[3]*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Saudi Arabia*
[#]*Equal Contribution*

Abstract

Osteoarthritis (OA) is the common form of chronic musculoskeletal degenerative joint disease in elder population and earlier studies have correlated the OA with genetics. The aim of this current study was analyse the collagen linked variants such as COL2A1 (rs73297147), CRTL1 (rs73771337), and CRTM (rs74063376) gene polymorphisms with clinically diagnosed and radiologically confirmed primary knee osteoarthritis in south Indian population. Two hundred genomic DNA samples were isolated from 100 primary knee OA cases and 100 healthy controls. Genomic DNA was isolated from peripheral blood sample and followed by PCR-RFLP analysis. A significant difference was observed in allele and dominant model in COL2A1 gene polymorphism (G vs A: p=0.01 (OR-1.10-2.48) OR=6.01) and (GG+AG: p=0.001 (OR-1.41-4.74) OR=2.6) within cases and controls. The T allele and TT+GT genotype of CRTL1 gene polymorphism was seen to be a significant difference (G: p=0.005 (OR-1.27-4.64) OR=2.43) and (TT+GT: p=0.01 (OR-1.2-4.74) OR=2.38). The CRTM polymorphism was negatively associated with all alleles and genotypes (p>0.05). GMDR interaction model supports the correlation among these 3 variants. The current results indicate that the rs73297147, rs73771337 polymorphisms in *COL2A1* and *CRTL1* genes could be associated with developing primary knee OA in south Indian population.

Keywords: Knee osteoarthritis; COL2A1; CRTL1; CRTM; Gene polymorphism

Abbreviations: OA: Osteoarthritis; GWAS: Genome Wide Association Studies; COL21: Collagen Type II Alpha 1; CRTL1: Cartilage Link Protein; CRTM: Cartilage Matrix Protein; SNP: Single Nucleotide Polymorphisms; PCR: Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphism; GMDR: Generalized Multifactor Dimensionality Reduction

Introduction

The osteoarthritis (OA) or degenerative joint disease is a progressive musculoskeletal disorder prone in elderly population. The disease OA itself is an active inflammatory condition and promises to remain a significant cause for pain and disability [1]. Age, genetics and environmental factors are the main risk factors for the cause of primary OA [2] and genetics is estimated to cause 30% risk for the development of the disease [3]. Knee OA is a mechanically driven disease with degenerative arthritis, proposed to be medial tibiofemoral knee-joint load increases with pharmacologic pain relief. The underlying mechanisms are still unknown and genetic factors contribute the development of OA the heritability has been estimated at 48%–65% [4].

The evidence of epidemiological studies proposes aging, genetic predisposition, obesity, inflammation and excessive mechanical loading predispose to OA development. Furthermore, the identification of candidate genes and pathways would help elucidate the molecular mechanisms of OA to have the potential to evaluate the risk for OA and lead to the development of OA gene-targeted therapies [5]. The genetic indication in OA cases originates from twin, family and animal model studies. Currently, the majority of genetic association studies on osteoarthritis disease risk focused on identifying the individuals effect on single nucleotide polymorphisms (SNPs) as well as their interaction effects on the disease. Based on genome-wide association studies (GWAS), twenty-one OA susceptibility loci have been established individually ($P \leq 5 \times 10^{-8}$) and with established functional association in OA from continuation studies [6,7]. Although some SNPs in numerous genes have been projected as OA susceptibility loci and few of them established in replication studies due to the genetic heterogeneity of OA. The documentation of candidate genes has essentially focused on encoding genes collagen, particularly type II collagen, and other structural proteins of cartilage extracellular matrix; bone and cartilage growth factors. Few genes deceive the occurrence and development of OA mainly regulate the formation, degradation and repair of articular cartilage or subchondral bone metabolism [8-13]. Genes predominantly encoding structural proteins of the extracellular matrix include collagen type II alpha 1 (*COL2A1*), cartilage link protein (*CRTL1*), and cartilage matrix protein (*CRTM*) genes that have been evaluated in the present study [11,14]. The aim of the current study was to analyze the collagen variants such as *COL2A1* (A28602G; rs73297147), *CRTL1* (G8206T; rs73771337), *CRTM* (A1412C; rs74063376) with clinically diagnosed knee osteoarthritis cases in south Indian population.

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Materials and Methods

Altogether, 200 South Indian individuals were recruited from Kamineni Hospitals, Hyderabad and India. The 100 primary knee OA cases were treated in the Department of Orthopedics and 100 healthy controls were recruited from master health checkup clinic. Adult OA cases were enrolled with the confirmation of clinical diagnosis and radiological score with 1-4 in Kellgren and Lawrence scale [15]. This case-control study was initiated with the institutional ethical approval and clinical, medical and family histories were documented in a well-designed proforma. The inclusion and exclusion criteria of both the cases/controls were informed in our prior studies [4,16]. We acquired 2 ml of the peripheral blood in an EDTA vacationer for extraction of genomic DNA from salting out technique, routinely used in our NABL accreditation laboratory [17]. Randomly 50 ul of Tris-EDTA buffer was used to solidify the genomic DNA and NanoDrop was used to measure the DNA quality (NanoDrop 2000, Thermo Fisher Scientific, MA, USA) [18]. Molecular analysis was performed by designing the specific primers for *COL2A1* (A28602G), *CRTL1* (G8206T), and *CRTM* (A1412C) candidate genes. Genotyping was done with the PCR and RFLP analysis. The protocol of the PCR has been described in our prior publications [19]. The PCR products were digested with precise restriction enzymes describes in Table 1 and followed by agarose gel electrophoresis.

Baseline clinical data are documented as mean ± standard deviation (M ± SD). The allele and genotypic associations between OA cases and controls were observed with odds ratio (OR) and 95% confidence interval followed by chi-square analysis. The middle-aged anthropometric, baseline and clinical characteristics has been documented in Table 1. Openepi software was used to accomplish the power calculation [20]. The genotypes for Hardy–Weinberg equilibrium (HWE) was intended by consuming OEGE (online encyclopedia calculator for genetic epidemiology studies) [21]. A p value of <0.05 was considered significant. Multifactor dimensionality reduction (MDR) and Generalized Multifactor dimensionality reduction (GMDR) model was implemented with prior study by Khan et al. [22].

Results

The present clinical data confirmed the negative association with gender (sex) and age ($p=0.88$ and $p=0.87$). The anthropometric information is organized in Table 2. The other body factors such as weight (0.001) and BMI (0.01) were associated in the predisposition of disease with the cases and controls. Furthermore, 28% of the cases had the family history of OA and 50% were undergoing treatment for hypertension. Genotypic and allelic distributions of all the polymorphisms satisfied HWE. Table 3 represents the proportion of allele and genotype frequencies in all the 3 polymorphisms in OA cases and controls. The allele data for *COL2A1* (A28602G) and *CRTL1* (G8206T) polymorphisms yielded positive association (P=0.01; OR=1.65, 95%CI 1.10–2.48; P=0.005; OR=2.43, 95%CI 1.27–4.64) and *CRTM* (A1412C) turns to be negative association (P=0.20; OR=2.38, 95%CI 0.60–9.34). Assessed polymorphisms for *COL2A1* (A28602G) and *CRTL* (G8206T) was associated with either genotype frequencies and or with dominant model between the cases and controls. The heterozygous genotypes in A28602G and G8206T polymorphisms were associated significantly (OR-2.66, P=0.02; 95%CI (1.41-4.99) and OR-2.27; 95% CI: 1.14-4.51), P=0.02). We observed the parallel similarity with the dominant model of genotype i.e. (GG+AG: $p=0.001$ (OR-1.41-4.74) OR=2.6; TT+GT: $p=0.01$ (OR-1.2-4.74) OR=2.38). For the polymorphism *CRTM* (A1412C), there was no evidence of the association either in allele form or genotype frequencies with the risk of OA, which was observed for rs74063376 polymorphism after yates' correction (P=0.2153; OR=2.235, 95%CI:0.609-8.199 and P=0.98; OR=1.04, 95%CI:0.02-53.1).

The genotype and allele frequencies of *COL2A1* (A28602G), *CRTL1* (G8206T), and *CRTM* (A1412C) polymorphisms was not associated within the gender stratification. However, Table 4 describes the statistical calculation for allele and genotype frequencies amongst the males and females of the OA patients.

One-three-way interaction of gene-gene model was listed in Table 5. In this study, general multi dimensionality reduction (GMDR) software was applied to discover the gene-gene interactions within the 3 SNPs. The current GMDR results confirms the single (rs73297147; P=0.05, Cross validation consistency was 10/10; testing balancing accuracy was

Gene	SNP	rs no	Band	Forward primer	Reverse primer	PCR Amplicon	Fragment Sizes (bp)
COL2A1	A28602G	rs73297147	12q13	TGAAGGAGAAGGTGTCTGCGGGA	GGACGGTGCGGTGAGAGTG	167 bp	A-167bp G-143/24 bp
CRTL1	G8026T	rs73771337	5q14	TCAGGCAGGAACAACACCAT	GGTTACTTCAAGGACAAAATACCTGTAAAGCT	249 bp	G-249 bp T-159/90 bp
CRTM	A1412C	rs74063383	1p35	CTGGAGACCACTCCCATCCTTTCT	GATGTGGCCATCACATTCGTCACGAT	237 bp	A-237 bp C-143/94 bp

Table 1: Candidate gene SNPs and primers used in this study.

Characters	Cases (n=100)	Controls (n=100)	p Value
Age (Years)	42.41 ± 8.11	42.17 ± 7.98	0.87
Sex: (M:F)	29:71	31:69	0.88
Height (cms)	156.63 ± 2.68	155.46 ± 2.45	0.37
Weight (kg)	76.97 ± 8.44	69.78 ± 7.87	**0.001**
BMI (kg/m²)	31.38 ± 3.4	25.89 ± 2.67	**0.01**
Age of Onset	39.52 ± 6.89	NA	NA
History of HTN	50 (50%)	15 (15%)	<0.01
History of T2DM	38 (38%)	14 (14%)	<0.01
History of Thyroid dysfunction	39 (39%)	22 (22%)	<0.01
NA=Not applicable			

Table 2: Clinical characteristics of OA cases and controls.

Genotypes	OA Cases	Controls	Odds ratio (95% CI)	χ2	*p* value
COL2A1(A28602G)					
AA	24 (24%)	45 (45%)			
AG	61 (61%)	43 (43%)	2.66 (1.41-4.99)	9.4	0.002
GG	15 (15%)	12 (12%)	2.34 (0.94 -5.80)	3.43	0.06
A	109 (0.545)	133 (0.665)			
G	91 (0.455)	67 (0.335)	1.65 (1.10-2.48)	6.01	0.01
CRTL1 (G8026T)					
GG	70 (70%)	85 (85%)			
GT	27 (27%)	15 (15%)	2.27 (1.14-4.51)	4.81	0.02
TT	03 (03%)	00 (0%)	8.48 (0.43-167.1)	2.79	0.09*
G	167 (0.835)	185 (0.925)			
T	33 (0.165)	15 (0.075)	2.43 (1.27-4.64)	7.65	0.005
CRTM (A1412C)					
AA	93 (93%)	97 (97%)			
AC	07 (07%)	03 (03%)	2.43 (0.61-9.7)	1.67	0.19
CC	00 (00%)	00 (00%)	1.04 (0.02-53.1)	0.0004	0.98*
A	193 (0.965)	197 (0.985)			
C	07 (0.035)	03 (0.015)	2.38 (0.60-9.34)	1.63	0.2
*Yates correction					

Table 3: Genotype and allele frequencies for the evaluated genes.

Genotypes	Females (n=71)	Males (n=29)	Odds ratio (95% CI)	*p* value
COL2A1 (A28602)				
AA	08 (11.3%)	16 (55.2%)		
AG	52 (73.2%)	09 (31%)	0.08 (0.02-0.26)	0.002
GG	11 (15.5%)	04 (13.8%)	0.18 (0.04-0.75)	0.01
A	68 (0.48)	41 (0.70)		
G	74 (0.52)	17 (0.30)	0.38 (0.2-0.7)	0.003
CRTL1 (G8026T)				
GG	47 (66.2%)	23 (79.3%)		
GT	22 (31%)	05(17.2%)	0.46 (0.15-1.38)	0.16
TT	02 (2.8%)	01 (3.5%)	1.0 (0.08-11.86)	0.98
G	116 (0.82)	51 (0.88)		
T	26(0.18)	7 (0.12)	0.61 (0.24-1.50)	0.28
CRTM (A1412C)				
AA	66 (93%)	27 (93.1%)		
AC	05 (7%)	02 (6 9%)	0.97 (0.17-5.35)	0.97
CC	00 (0%)	00 (0%)	2.41 (0.04-124.9)	0.65
A	137 0.97)	56 (0.965)		
C	05 (0.03)	02 (0.035)	0.97 (0.18-5.2)	0.97

*Yates correction

Table 4: Genotype and allele frequencies of OA males and OA females.

60.3%) and three-locus (rs73297147 rs73771337 rs74063376; P=0.05, Cross validation consistency was 10/10; testing balancing accuracy was 58.5%) model as the positive association in the OA disease. However, we could find the significant association with two-locus (rs73297147 rs73771337; P=0.17, Cross validation consistency was 05/10; testing balancing accuracy was 57.5%) of GMDR model.

An entropy graph obtained after MDR analysis showed interactions of gene polymorphisms with other anthropometric parameters and clinical features in primary knee OA compared to controls. BMI (14.09%), type 2 diabetes (5.11%), hypertension (9.89%), and thyroid dysfunction (2.24%) contributed to primary knee OA. BMI contributed most to disease pathology and has strong synergistic interaction with the COL2A1 G allele with an entropy of 2.83%, redundancy with hypertension and thyroid dysfunction (Figure 1).

Discussion

Genetic association of *COL2A1* (A28602G), *CRTL1* (G8206T) and *CRTM* (A1412C) polymorphic variants with primary knee OA risk and aggressiveness were investigated in this hospital based case-control study in South Indian population. The variants for A28602G and G8206T were associated with AG and GT genotypes appeared 2.3 and 2.2 fold increased risk for primary knee OA. However, we could not identify the significant difference with A1412C genotype frequency within the cases and controls. Also, there was no gender difference between the OA cases and controls in three gene polymorphisms.

OA has converted as joint disorder and a leading cause of disability in elderly population and the early onset of OA has prone to be major risk issue in knee OA [23,24]. The involvement between genetics and

BMI	OA Cases (n=100)	BMI (Mean ± SD)	Genotypes-COL2A1		
			AA	AG	GG
Normal (0-24.9)	6	23.42 ± 1.19	1(16.7%)	4(66.6%)	1 (16.7%)
Overweight (25.0-29.9)	24	28.56 ± 0.94	5(20.8%)	12 (50%)	7 (29.2%)
Obese (>30.0)	70	33.18 ± 3.40	10(14.3%)	52(74.3%)	8 (11.4%)
	OA Cases (n=100)	BMI (Mean±SD)	Genotypes-CRTL1		
			GG	GT	TT
Normal (0-24.9)	6	23.42 ± 1.19	5(83.3%)	1(16.7%)	0 (0%)
Overweight (25.0-29.9)	24	28.56 ± 0.94	19(79.2%)	4 (16.7%)	1(4.1%)
Obese (>30.0)	70	33.18 ± 3.40	43(61.4%)	25(35.7%)	2 (2.9%)
	OA Cases (n=100)	BMI (Mean±SD)	Genotypes-CRTM		
			AA	AC	CC
Normal (0-24.9)	6	23.42 ± 1.19	6(100%)	(0%)	0 (0%)
Overweight (25.0-29.9)	24	28.56 ± 0.94	22(91.7%)	2 (8.3%)	0 (0%)
Obese (>30.0)	70	33.18 ± 3.40	66(94.3%)	4(5.7%)	0 (0%)

Table 5: Association between BMI and genotypes of specific cartilage genes component-encoding genes evaluated in this study.
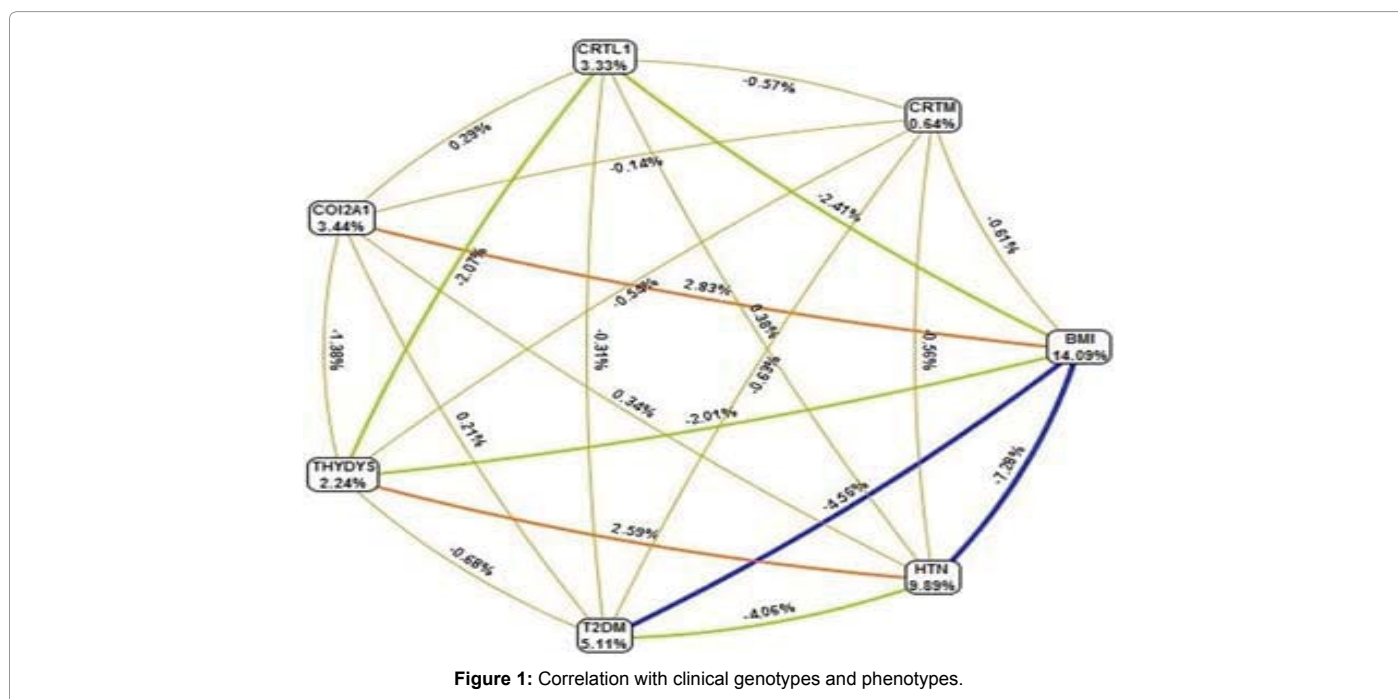

Figure 1: Correlation with clinical genotypes and phenotypes.

OA has been broadly scrutinized in linkage, association and family based studies. Genetic association studies have helped in recognizing the effects of precise gene variants on OA pathogenesis, but had only minor effects specifying the complex polygenic nature of OA [25]. The exposure of OA is coded with DNA polymorphism and as such these studies involved a comparison of the frequency of polymorphisms in individuals with the disease versus those with disease-free controls, or those in whom osteoarthritis status had not been determined. Such case-control studies have tremendous power, so long as certain criteria are encountered [26].

The selection of *COL2A1* candidate gene is due to the effect of protein in the articular cartilage. Mutations in *COL2A1* affect joint function, bone development and cause hereditary cartilage diseases and type II collagen encodes the *COL2A1* protein, most abundant in articular cartilage and intervertebral disk, an additional vital protein elaborated with expansion of OA [27]. Meulenbelt et al. [28] was the initial study conceded the positive association with *COL2A1* gene with hip and knee OA in Rotterdam study. Earlier studies on *COL2A1*

association with OA and knee OA were conducted in populations of different ethnicities [11,27-31].

The cartilage link proteins such as CRTL1 and CRTM stabilizes proteoglycan aggregates through binding both the proteoglycan core protein and to hyaluronic acid [11]. The CRTL1 protein appears in the non-collagenous extracellular matrix also contributes the etiology of OA disease. The gene *CRTL1* stabilizes proteoglycan aggregates by binding to both the proteoglycan core protein and hyaluronic acid [32,33]. CRTM encodes a major protein component appear in the non-articular cartilage of the extracellular matrix fixes to collagen. The mutation G8206T (rs7772941) in *CRTM* have been associated with variety of inherited chondrodysplasias. *CRTM* polymorphisms were not associated with primary knee OA (A1412C: OR=2.43, 95% CI=0.61–9.7, p=0.19 and OR=2.38, 95% CI=0.60–9.043, p=0.20). Meulenbelt et al. [11] conducted a study on associations in Rotterdam population between CRTM and CRTL1 and generalized OA, and concluded as *CRTM* locus may play a role in OA in a gender- and joint site-specific manner. Our results were not in agreement with those of

Meulenbelt et al. [11,28], as our results showed an association of OA with *CRTL1* but not with *CRTM*. This may be due to differences in ethnicity of studied populations and the BMI, a well-established risk factor for weight gain in patients with OA.

We have categorized OA cases based on BMI into (i) 18.5–24.9 BMI as normal, (ii) 25.0–29.9 BMI as overweight and (iii) >30 BMI as obese groups as BMI is one of the major factor contributing for the disease pathology. The mean BMI for normal and overweight groups were 23.42 ± 1.19 and 28.56 ± 0.94, respectively. The mean BMI for the obese group was 33.18 ± 3.40 (Table 5). The prevalence of obesity in the AG genotype of the *COL2A1* (A28602G) polymorphism was 74.3%, whereas that in GG and AA genotypes of *CRTL1* (G8206T) and *CRTM* (A1412C) polymorphisms was 61.4% and 94.3%, respectively. Homozygous (AA), heterozygous, and obese variant genotypes of *COL2A1* contribute significantly to the heritability of primary knee OA. From our analysis, we detected around 70% of abnormal BMI patients detected with G and T alleles in *COL2A1* and *CRTL1*, respectively. However, the OA patients with <25 kg/m² BMI possessed deleterious alleles. The *ANOVA* analysis revealed the significant association between BMI and genotypes ($p<0.05$). The prior study from our group within the OA patients exhibited the positive association with *ACE* I28005D polymorphism [16]. This study accomplishes that obese patients are at a higher risk for OA in south Indian population.

Gene-Gene and Gene-environment connections were analyzed with MDR statistical tool, a method used for combining high dimensional genetic data into a single dimension, which permits interactions to be detected in relatively small samples [22]. MDR analysis was conducted to find interactions among genotypes of OA cases and controls with demographic parameters. The results of MDR indicated that *COL2A1* (rs73297147) and combination of three SNPs i.e., (rs73297147+rs73771337+rs74063376) contribute to pathogenesis of OA and discloses strong interaction.

The limitations of our present study comprises that we have analyzed only one SNP from each gene. In conclusion, our study showed that A28602G and G8206T polymorphisms in *COL2A1* and *CRTL1* genes were significantly associated with primary knee OA in the South Indian population.

Acknowledgement

Conflict of Interest

There is no conflict of Interest towards this article.

References

1. Freitag J, Bates D, Boyd R, Shah K, Barnard A, et al. (2016) Mesenchymal stem cell therapy in the treatment of osteoarthritis: reparative pathways, safety and efficacy - a review. BMC Musculoskelet Disord 17: 230.

2. Sandell LJ (2012) Etiology of osteoarthritis: genetics and synovial joint development. Nat Rev Rheumatol 8: 77-89.

3. Warner CS, Valdes AM (2016) The genetics of osteoarthritis: A review. Journal of Functional Morphology and Kinesiology 1: 140-153.

4. Subramanyam K, Poornima S, Juturu KK, Anand D, Mohanthy S, et al. (2016) Missense FokI variant in the vitamin D receptor gene in primary knee osteoarthritis patients in south Indian population. Gene reports 4: 118-122.

5. Wang Y, Chu M, Rong J, Xing B, Zhu L, et al. (2016) No association of the single nucleotide polymorphism rs8044769 in the fat mass and obesity-associated gene with knee osteoarthritis risk and body mass index: A population-based study in China. Bone Joint Res 5: 169-174.

6. Aury-Landas J, Marcelli C, Leclercq S, Boumédiene K, Baugé C (2016) Genetic Determinism of Primary Early-Onset Osteoarthritis. Trends Mol Med 22: 38-52.

7. Ramos YF, Meulenbelt I (2015) Implementation of Functional Genomics for Bench-to-Bedside Transition in Osteoarthritis. Curr Rheumatol Rep 17: 53.

8. Lv C, Li Y, Xu J, Cao H, Li X, et al. (2015) Association of SPP1 promoter variants with hip osteoarthritis susceptibility in Chinese population. Gene 564: 9-13.

9. Chapman K, Valdes AM (2012) Genetic factors in OA pathogenesis. Bone 51: 258-264.

10. Meulenbelt I, Bijkerk C, Breedveld FC, Slagboom PE (1997) Genetic linkage analysis of 14 candidate gene loci in a family with autosomal dominant osteoarthritis without dysplasia. J Med Genet 34: 1024-1027.

11. Meulenbelt I, Bijkerk C, de Wildt SC, Miedema HS, Valkenburg HA, et al. (1997b) Investigation of the association of the CRTM and CRTL1 genes with radiographically evident osteoarthritis in subjects from the Rotterdam study. Arthritis Rheum 40: 1760-1765.

12. Zheru D, Peiliang F, Yuli W, Haishan W, Qirong Q, et al. (2014) Association of PPARÎ³ gene polymorphisms with osteoarthritis in a southeast Chinese population. J Genet 93: 719-723.

13. Liying J, Yuchun T, Youcheng W, Yingchen W, Chunyu J, et al. (2013) A SMAD3 gene polymorphism is related with osteoarthritis in a Northeast Chinese population. Rheumatol Int 33: 1763-1768.

14. Loughlin J (2001) Genetic epidemiology of primary osteoarthritis. Curr Opin Rheumatol 13: 111-116.

15. Kellgren JH, Lawrence JS (1957) Radiological assessment of osteo-arthrosis. Ann Rheum Dis 16: 494-502.

16. Poornima S, Subramanyam K, Khan IA, Hasan Q (2015) The insertion and deletion (I28005D) polymorphism of the angiotensin I converting enzyme gene is a risk factor for osteoarthritis in an Asian Indian population. J Renin Angiotensin Aldosterone Syst 16: 1281-1287.

17. Khan IA, Movva S, Shaik NA, Chava S, Jahan P, et al. (2014) Investigation of Calpain 10 (rs2975760) gene polymorphism in Asian Indians with Gestational Diabetes Mellitus. Meta Gene 2: 299-306.

18. Khan IA, Shaik NA, Kamineni V, Jahan P, Hasan Q, (2015) Evaluation of Gestational Diabetes Mellitus Risk in South Indian Women Based on MTHFR (C677T) and FVL (G1691A) Mutations. Front Pediatr 3: 34.

19. Khan IA, Vattam KK, Jahan P, Hasan Q, Rao P (2016) Importance of glucokinase -258G/A polymorphism in Asian Indians with post-transplant and type 2 diabetes mellitus. Intractable Rare Dis Res 5: 25-30.

20. Etokebe GE, Jotanovic Z, Mihelic R, Mulac-Jericevic B, Nikolic T, et al. (2015) Susceptibility to large-joint osteoarthritis (hip and knee) is associated with BAG6 rs3117582 SNP and the VNTR polymorphism in the second exon of the FAM46A gene on chromosome 6. J Orthop Res 33: 56-62.

21. Nikkari ST, Määttä KM, Kunnas TA (2015) Functional Inducible Nitric Oxide Synthase Gene Variants Associate with Hypertension: A Case-Control Study in a Finnish Population-The TAMRISK Study. Medicine (Baltimore) 94: e1958.

22. Khan IA, Jahan P, Hasan Q, Rao P (2015) Validation of the association of TCF7L2 and SLC30A8 gene polymorphisms with post-transplant diabetes mellitus in Asian Indian population. Intractable Rare Dis Res 4: 87-92.

23. Goldring MB, Berenbaum F (2015) Emerging targets in osteoarthritis therapy. Curr Opin Pharmacol 22: 51-63.

24. Driban JB, Morgan N, Price LL, Cook KF, Wang C, et al. (2015) Patient-Reported Outcomes Measurement Information System (PROMIS) instruments among individuals with symptomatic knee osteoarthritis: a cross-sectional study of floor/ceiling effects and construct validity. BMC Musculoskelet Disord 16: 253.

25. Yucesoy B, Charles LE, Baker B, Burchfiel CM (2015) Occupational and genetic risk factors for osteoarthritis: a review. Work 50: 261-273.

26. Loughlin J (2015) Genetic contribution to osteoarthritis development: current state of evidence. Curr Opin Rheumatol 27: 284-288.

27. Zhai G, Rivadeneira F, Houwing-Duistermaat JJ, Meulenbelt I, Bijkerk C, et al. (2004) Insulin-like growth factor I gene promoter polymorphism, collagen type II alpha1 (COL2A1) gene, and the prevalence of radiographic osteoarthritis: the Rotterdam Study. Ann Rheum Dis 63: 544-548.

28. Meulenbelt I, Bijkerk C, De Wildt SC, Miedema HS, Breedveld FC, et al.

(1999) Haplotype analysis of three polymorphisms of the COL2A1 gene and associations with generalised radiological osteoarthritis. Ann Hum Genet 63: 393-400.

29. Knowlton RG, Katzenstein PL, Moskowitz RW, Weaver EJ, Malemud CJ, et al. (1990) Genetic linkage of a polymorphism in the type II procollagen gene (COL2A1) to primary osteoarthritis associated with mild chondrodysplasia. N Engl J Med 322: 526-530.

30. Valdes AM, Loughlin J, Oene MV, Chapman K, Surdulescu GL, et al. (2007) Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. Arthritis Rheum 56: 137-146.

31. Valdes AM, Hart DJ, Jones KA, Surdulescu G, Swarbrick P, et al. (2004) Association study of candidate genes for the prevalence and progression of knee osteoarthritis. Arthritis Rheum 50: 2497-2507.

32. Lockhart M, Wirrig E, Phelps A, Wessels A (2011) Extracellular matrix and heart development. Birth Defects Res A Clin Mol Teratol 91: 535-550.

33. Urano T, Narusawa K, Shiraki M, Sasaki N, Hosoi T, et al. (2011) Single-nucleotide polymorphism in the hyaluronan and proteoglycan link protein 1 (HAPLN1) gene is associated with spinal osteophyte formation and disc degeneration in Japanese women. Eur Spine J 20: 572-577.