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Role of *SREBP2* gene polymorphism on knee osteoarthritis in the South Indian Hyderabad Population: A hospital based study with G595C variant

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ABSTRACT

Introduction: Osteoarthritis (OA) is a multifactorial disease with genetic factors playing a crucial role, and it has been associated with a family history of obesity. G595C polymorphism in the sterol regulatory element-binding protein 2 (*SREBP2*) gene has demonstrated an association with knee osteoarthritis (KOA) patients. However, this polymorphism has been never explored in an Indian population. Hence, the current study aimed to examine whether G595C (rs2228314) polymorphism in *SREBP2* gene was associated with KOA susceptibility in the South Indian Hyderabad population.

Methods: G595C polymorphism was genotyped with 200 KOA cases and 200 healthy controls using polymerase chain reaction-restriction fragment length polymorphism analysis.

Results: A significant association was observed between age, body mass index (BMI), and family histories in KOA cases and controls ($p < 0.05$). The current allele (C vs G; OR-2.8 [95%CI = 2.1–3.7]; $p < 0.0001$) and genotype analysis confirms the significant association with (GC + CC vs GG; OR-3.5 [95%CI = 2.3–5.3]; $p < 0.0001$ & GC vs GG + CC; OR-1.7 [95%CI = 1.0–2.9]; $p = 0.02$) KOA vs. control subjects. On stratification analysis, genotype CC and C allele were associated with KOA. Gender association failed to demonstrate positive genotype frequencies ($p > 0.05$). Multifactor-dimensionality reduction (MDR) analysis showed a positive association with BMI and G595C genotypes ($p < 0.05$); 51% of the homozygous variant CC genotypes were present in obesity subjects.

Conclusion: In conclusion, our findings suggest that G595C polymorphism in *SREBP2* gene is associated with KOA in the South Indian Hyderabad population and presents scope for further investigation of the gene's function in KOA.

1. Introduction

Osteoarthritis (OA; OMIM 165720) is documented as the basic form of arthritis and can originate from progressive loss of joint function.[1] Disjoint of hip and knee leads to the major cause of pain, functional loss, and poor quality of life.[2] It is also documented as a late-onset musculoskeletal disease in the elder population, characterized by gradual degradation of the articular cartilage by additional synovium injury, subchondral, and other types of bone-joint tissue.[3] Knee osteoarthritis (KOA), a type of degenerative arthritis, results from breakdown of cartilage and underlying bone. Knee pain is the most common feature in KOA, and 10% of disability ensues in the elder population (> 55 Years).[4] The prevalence of KOA increases with obesity and age.[5] Morphological, biochemical, molecular, and biomedical changes in the extra-cellular matrix and cells are commonly noted in OA, which is a collective result of the genetic and environmental factors, while 50% of genetic factor results are prone for OA disease.[6] This disease initially effects the knees and joint cartilage with loss of function in 9.6% in men and 18% in women. Age, gender, sex, obesity, race, genetics, diet, and injury in joints and bones are considered as risk factor of OA. Twin and family studies are largely associated with undocumented genetic variants.[7] Heritable studies in OA have identified gene variants involved in cartilage degradation.[8] Many attempts have been made to document the radiographic grade of disease in OA, which is widely used in

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assessing the Kellgren and Lawrence (K&L) score. The overall grades of severity are determined from 0 to 4 and are related to the sequential appearance of osteophytes, joint space loss, sclerosis, crepitus, and cysts.[9] The estimated heritability of primary OA is as high as 40% for the knee, 60% for the hip, and 65% for the hand.[10] The progression of OA is very slow and increases slightly within years to decade. No effective treatment strategy is available as yet.[11] Numerous studies have found an association between obesity and OA/KOA.[12] Both biomechanical and metabolic factors are interrelated with obesity in OA; biomechanical overload on the joints can accelerate cartilage degeneration, while metabolic dysfunction triggered by obesity is related to OA.[13] Genetic polymorphism has been useful for identification of numerous monogenetic disorders and similar strategy has been applied for complex diseases, such as asthma, cancer, heart disease, and OA. Single nucleotide polymorphisms (SNPs) are the most common genomic DNA variations within any population.[14] Simultaneously, genome-wide association studies (GWAS) is susceptibility known for OA, which is influenced by genetic predisposition.[15] In KOA, the GWAS have documented *PRKAR2B*, *HPB1*, *COG5*, *GPR22*, *COG5*, *GDF5*, and *ADAM12* polymorphisms.[16]

Sterol regulatory element-binding protein 2 (SREBP2; OMIM-600481) is defined as the member of SREBPs nuclear transcription factor family, which binds with the sterol regulator in the promoter or enhancer in the lipid synthetic enzyme gene. It activates the target gene transcription as well as gene expression of the cholesterol biosynthesis pathway, which plays a key role in lipid homeostasis.[17] *SREBP2* gene is associated with multiple metabolic disorders, such as obesity, metabolic syndrome, and Body mass index (BMI).[18–22] Previous studies were concerned mostly with obesity, and there are limited studies conducted in OA.[18] Our earlier studies have documented the genetic studies with OA.[23,24] Hence, the current case-control study was investigated for G595C polymorphism in the *SREBP2* gene in patients with KOA in the South Indian Hyderabad population.

2. Materials and methods

2.1. Sample analysis

All patients and controls were recruited from the Department of Orthopedics, (Kamineni and Yashoda) Hospitals, Hyderabad, India. As per a prior study,[23] we have increased our sample sizes to 200 KOA cases and 200 healthy controls. The inclusion criteria of the KOA cases were based on the clinical and radiological diagnosis of primary osteoarthritis as per the Kellegren/Lawrence score (0–4 score),[9] which was described by Subramanyam et al..[24] The age range of the KOA cases was below 55 years. We excluded other knee diseases, such as post-septic, rheumatoid, and post-traumatic arthritis in the cases. We recruited age and gender matched controls from the healthy population without a family history of OA, obesity, and musculoskeletal diseases from the master health checkup.[23] We have already approved the ethical grant for this study as per the principle of Helsinki declaration.[25] All the patients who participated in this study provided a written consent form and completed a questionnaire. Based on the WHO criteria, BMI was measured in both the males and females.

2.2. Blood analysis

In an EDTA vacutainer, 2 mL of the peripheral blood was collected from 400 participants, and using the salting out technique, DNA extraction was performed in an NABL accredited laboratory,[26] Department of Genetics and Molecular Medicine, Kamineni hospitals, Hyderabad, India. To check the purity of the DNA, we used the NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fischer Scientific, MA, USA) and stored the samples at −20 °C for further usage. The SNP rs2228314 (G595C) in *SREBP2* gene, located at exon 10, was genotyped using the thermal cycler polymerase chain reaction (PCR), followed by restriction fragment length polymorphism (RFLP) analysis with sense and antisense primers supplied by Bioserve Biotechnologies, Hyderabad, India. The details of the amplification reaction and PCR conditions were described in our prior publication.[24,26]

The NaeI restriction enzyme (Thermo Scientifics, USA) was digested using the 245bp PCR product at 37 °C at 18 h for conversion into the normal allele i.e., 151, 51 and 37 bp and the mutant allele was the 245bp. Digested (3%) and undigested (2%) PCR products were run on the concerned percentages of agarose gel and the results were visualized using the Kamineni gel documentation system (Kamineni Life Sciences Pvt Ltd, Hyderabad, India). Approximately, 10% of samples were repeated and we found a concordance rate of 100%.

2.3. Statistical analysis

Clinical data was calculated between KOA cases and controls with *t-tests*. Openepi software was used to calculate the statistical analysis. Hardy-Weinberg Equilibrium was calculated with G595C variant. Genotype analysis with Odds ratios (ORs), 95% Confidence Intervals (CIs) and p values were calculated between cases and controls. MDR and Anova analysis were calculated. P value less than 0.05 were considered as significant association ($p < 0.05$).

3. Results

3.1. Assessment of baseline characteristics

Altogether, 400 subjects were evaluated in this hospital based case-control study. The KOA patients comprised 81 (40.5%) males and 119 (59.5%) females. The healthy controls comprised 86 (43%) males and 114 (57%) females. The demographic details are presented in Table 1. The mean height of the KOA subjects was 155.1 ± 4.5 cms and that of the controls was 155.9 ± 3.9 cms ($p = 0.04$). The mean BMI of the cases was 30.4 ± 3.8 kg/m^2 and in controls it was 25.5 ± 3.3 kg/m^2 indicating KOA cases had higher percentage of obesity ($p = 0.0005$). However, the family history of KOA was found to be 28.5% in the KOA cases. The statistical analysis was calculated between KOA cases and controls and we found that the height, weight, BMI and family histories had a significant association ($P < 0.05$).

3.2. Genotyping of NaeI site

Three genotypes were detected with *Nae*I restriction enzyme: GG (151 + 51 + 37bp), GC (245 + 151 + 51 + 37bp), and CC (241bp). G/C single nucleotide polymorphism with *Nae*I enzyme was observed. The rs2228314 polymorphism in the *SREBP2* gene met the HWE ($p > 0.05$). Genotype and allele frequencies of rs2228314 polymorphism with KOA cases and healthy controls are presented in Table 2. A strong significant association was documented between the allele and genotype analysis. The genotype frequency for rs2228314 polymorphism was found to be 58% for GG, 25.5% for GC, and 45.5%

Table 1
Demographic characteristics of primary KOA subjects and healthy controls.

Characters	Cases ($n = 200$)	Controls ($n = 200$)	p Value
Age (Years)	46.02 ± 8.02	44.23 ± 6.78	0.04
Sex: (M: F)	81 : 119	86 : 114	NA
Height (cms)	155.13 ± 4.54	155.59 ± 3.94	0.04
Weight (kg)	73.27 ± 9.64	61.81 ± 7.53	0.0005
BMI (kg/m^2)	30.44 ± 3.81	25.57 ± 3.30	0.03
Age of Onset	41.12 ± 6.30	NA	NA
Family History of OA	57 (28.5%)	NA	NA
History of HTN	91 (45.5%)	34 (17%)	0.0007
History of T2DM	60 (30%)	26 (13%)	0.0002
History of Thyroid Dysfunction	50 (25%)	28 (14%)	0.0001

NA = Not Applicable.

Table 2
Genotype and allele distribution of *SREBP2* (G595C) gene in KOA cases.

rs number	Model	Genotypes	Cases	Controls	OR (95% CI)	p Value
rs2228314 (SREBP2)	Normal	GG	58 (29%)	118 (59%)	**Reference**	
	Heterozygous	GC	51 (25.5%)	32 (16%)	3.24 (1.88–5.57)	p < 0.0001
	Variant	CC	91 (45.5%)	50 (25%)	3.7 (2.32–5.90)	p = 0.001
	Dominant	CC + GC vs GG	142 (71%)	82 (41%)	3.52 (2.32–5.33)	p < 0.0001
	Co-dominant	GC vs CC + GG	51 (25.5%)	32 (16%)	1.79 (1.09–2.94)	p = 0.02
	Recessive	CC vs GC + GG	91 (45.5%)	50 (25%)	2.50 (1.63–3.82)	p < 0.0001
		G	167 (0.4175)	268 (0.67)	**Reference**	
	Risk	C	202 (0.5825)	132 (0.33)	2.83 (2.12–3.77)	p < 0.0001

for CC in the KOA cases and 59% in GG, 16% in GC, and 25% in CC in the healthy controls (GC vs GG-OR-3.2, 95%CI = 1.8–5.5; p < 0.0001 and CC vs GG-OR-3.7, 95%CI = 2.3–5.9; p = 0.001). The dominant, co-dominant and variant genotypes showed strong association (CC + GC vs GG-OR-3.5, 95%CI = 2.3–5.3; p < 0.0001; GC vs GG + CC-OR-1.7, 95%CI = 1.1–2.9; p = 0.02 and CC vs GC + GG OR-2.5, 95%CI = 1.6–3.8; p < 0.0001). The allele frequencies for G and C in the KOA cases were 42% and 58%, respectively, while those in the healthy controls were 67% and 33%, respectively (C vs. G-OR-2.8, 95%CI = 2.1–3.7; p < 0.0001).

3.3. Gender stratification

The allele and genotype frequencies between male and female subjects in the KOA cases for G595C polymorphism are described in Table 3. The genotype frequencies for GG, GC, and CC are 28%, 26%, and 46% in the males (n = 81), respectively, and 29%, 25%, and 46%, respectively in the females (n = 119). There was no significant difference between any of the alleles, genotype frequencies, or dominant, co-dominant, and recessive models of inheritance (p > 0.05).

3.4. Correlation between G595C genotypes and BMI

Genotype frequencies were categorized into three categories: normal (n = 15), overweight (n = 76), and obesity (n = 109) as presented in Table 4. The percentage of genotype frequencies in the obesity cases were GG (59%), GC (56%), and CC (51%) and those in the overweight cases were GG (36%), GC (39%), and CC (40%).

3.5. Multifactor-dimensionality reduction analysis

The multifactor-dimensionality reduction (MDR) analysis was carried out to analyze the relation between gene polymorphism and the co-morbidity factor interactions. The current interactive graph indicates that SREBP2 (G595C) contributes 6.7% towards the disease pathology (Fig. 1). Co-morbidity factors, such as BMI, hypertension, type 2 diabetes, and thyroid dysfunction parameters contributed 23.0%, 7.0%, 3.1%, and 1.4%, respectively towards the disease pathology and showed an association between BMI and primary KOA. The relation between G595C polymorphism and BMI also disclosed a synergistic interaction with thyroid dysfunction with an entropy value of 5.2%.

4. Discussion

The aim of this study was to investigate the impact of G595C polymorphism in the *SREBP2* gene on KOA in the South Indian Hyderabad population. To the best of our knowledge none of the studies have documented *SREBP2* gene polymorphism in the Indian population. Our study, conducted on elderly patients with KOA from South Indian Hyderabad population showed genetic association with the G595C variant. The C allele demonstrated a 2.8-fold risk in the KOA patients (p = 0.0001). The G595C polymorphism was significantly associated with BMI in OA patients (p < 0.05) and results of this study (Table 3) conclude that the *Nae*I site is not associated with gender specific polymorphism. KOA is the main cause of disability and the disease itself appear in the entire joint of the elderly population.[27] Cases with KOA are expected to increase due to the global aging population and obesity.[28] The combination of increase in age with obesity affects the OA patients. A significant association was documented between the various genetic variants in the OA patients and BMI.[29–31] The overweight and obesity in the middle-aged population were recognized as risk factors for KOA and limited studies have concluded that excess weight gain in childhood causes knee pain and develops into OA in the middle-age.[32,33] Other studies have concluded that the excess weight in the adult population plays an important role in the risk of KOA and joint replacement.[34,35] Holliday et al.[36] states that BMI was found to be a strong risk factor for KOA and in our study the MDR results confirmed positive association (23.4%) between BMI and homozygous variant (CC) genotypes in overweight and obese patients with KOA.

SREBP2 gene is present on the chromosome region at 22q13.2. The SNP rs2228314 (G595C) is located in the exon 10 region and substitutes the amino acids from glycine with alanine at 595th position in the SREBP2 protein37. SREBP2 is expressed in the transcription factor that plays an important role in the regulation of cholesterol and fatty-acid metabolism, and *SREBP2* gene controls cholesterol homeostasis by stimulating transcription of the sterol regulated genes.[38] The protein from the SREBP2 is encoded preferentially for activating the genes of cholesterol metabolism.[39] Initially, SREBPs were isolated from *in vitro* human hela cells and this study has concluded the correlation between *SREBP2* gene and low density lipoprotein receptor.[40] Yang et al.[41] initially documented the mutation in the *SREBP2* gene, and Muller et al.[22] provided the evidence for the mutations in *SREBP2* gene associated with hypercholesterolemia in humans. The mutation G1748C is mainly

Table 3
Statistical association between male and female genotypes in the primary KOA cases.

rs number	Model	Genotypes	Male	Female	OR (95% CI)	p Value
rs2228314 (SREBP2)	Normal	GG	23 (28%)	35 (29%)	Reference	
	Heterozygous	GC	21 (26%)	30 (25%)	3.24 (1.88–5.57)	p = 0.0001
	Variant	CC	37 (46%)	54 (46%)	3.70 (2.32–5.90)	p = 0.0001
	Dominant	CC + GC vs GG	44 (80%)	71 (75%)	1.05 (0.56–1.96)	p = 0.87
	Co-dominant	GC vs CC + GG	21 (26%)	30 (25%)	1.03 (0.54–1.98)	p = 0.90
	Recessive	CC vs GC + GG	37 (46%)	54 (46%)	1.01 (0.57–1.78)	p = 0.96
		G	67 (0.41)	100 (0.42)	Reference	
	Risk	C	95 (0.59)	138 (0.58)	1.02 (0.68–1.54)	p = 0.89

Table 4
Variance with BMI and genotypes of *SREBP2* (G595C) gene.

	OA Cases (n = 200)	BMI (Mean ± SD)			
			GG	GC	CC
Normal (0–24.9)	15	23.12 ± 2.51	3 (5%)	3 (5%)	8 (9%)
Overweight (25.0–29.9)	76	27.82 ± 1.68	21 (36%)	20 (39%)	36 (40%)
Obese (> 30.0)	109	33.10 ± 3.67	34 (59%)	28 (56%)	47(51%)

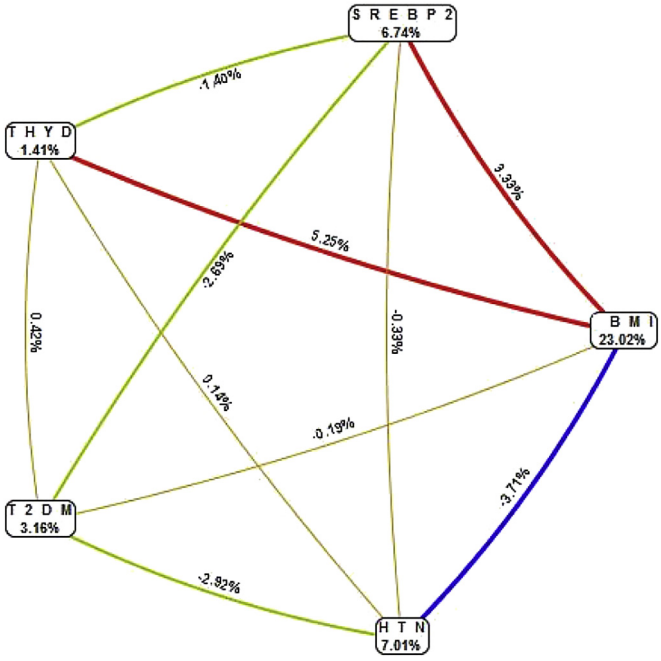

Fig. 1. Entropy graph indicating SREBP2 gene polymorphism and co-morbid factor interactions.

connected with metabolic syndrome, and G595C variant with atherosclerosis, hypercholesterolaemic, eminent plasma levels, and obesity.[18,22,42] Limited genetic studies have been implemented in KOA and G595C polymorphism in the global population.[18,22,37,42,43] Furthermore, the inhibition of the integrins in OA leads to SREBP2 and MMP-13 down regulation with subsequent elevation of the activated aggrecan levels, suggesting integrin blockage as a potential molecular target for OA treatment. Primarily, rs2228314 polymorphism was identified through the cohort study in the Greek population as a risk factor for OA.[43] The maximum studies of G595C polymorphism have been carried out in the Chinese population with different diseases, such as OA, premature coronary artery disease, coronary artery disease, fatty liver disease, polycystic ovarian syndrome, and obesity.[18,37,42–44] Our study was the initial study documented in the Indian population with a strong association.

Genetic epidemiology and genetic risk factors can influence the risk of OA and KOA at numerous stages during the progression of the disease. Genetic variants might influence the OA disease and risk factors like, skeletal shape, obesity, synovitis, and bone mass.[45] Molecular genetic investigations have gained significant knowledge regarding the OA/KOA etiology and have documented the evidence with the genetic component to OA.[46] With the help of GWAS, many genetic loci were confirmed and have particular relevance for the disease progression in precise skeletal sites and additionally, few of the genetic and non-genetic loci are related with OA disease in the global ethnicities.[47–49] Genetic polymorphisms are elaborated in biodiversity, which could be predisposed by ethnic heritage and geographic localization.[50] G595C polymorphism has received detailed consideration since the last decade. This polymorphism is indirectly connected through cholesterol to the metabolic-disorder to obesity and then with OA to KOA. We enrolled 54.5% of obesity subjects and 38% of overweight subjects in this study. This polymorphism was in accordance with several studies carried out with different diseases in the global population. At present, there is no treatment for OA and KOA. Basically, this disease is graded into five categories - normal, minor, mild, moderate, and severe. However, the diseases are treated using supplements, exercises, laboratory reports, radiographs, cortisone injections, bone surgery, total knee replacement, or arthroplasty. The earlier studies have documented and discussed the connection between OA and genetics through BMI (obesity).[23,24] Many genetic studies have been carried out with GWAS, case-control, hospital based studies, retrospective, prospective, meta-analysis, next-generation sequencing, exome sequencing, and large cohort population. Zengini et al.[51] reported a recent GWAS study in OA with 16.5 million variants through the UK biobank resource i.e., (297,191 controls and 30,272 cases). Altogether, 9 new genetic loci were documented in the non-coding region. These documented OA loci are related to BMI. Yang et al.[52] introduced traditional Chinese medicine (TCM) for patients suffering with KOA and observed potential benefits of TCM in patients with KOA in the Chinese population. However, clinical trials are not confirmed to implement these methods in the global population.

Since the last decade, meta-analysis studies have confirmed the accuracy of the results of prior studies conducted on the global population.[26] However, G595C polymorphism was not documented with any meta-analysis studies in any of the specific diseases. This could be one of the limitations for this polymorphism. However, many meta-analyses were documented with KOA disease.[53,54] Selection of KOA patients was the strength of this study. Opting single polymorphism and skipping of validation were the limitations of this study.

5. Conclusion

This study concludes that *SREBP2* gene (G595C) polymorphism in associated with KOA in the South Indian Hyderabad population. Such polymorphisms can explain the high risk of the subjects with these diseases. Our study recommends that a similar study be conducted in a large population of KOA patients globally to validate the results of the present study and deliver more information on the G595C polymorphism in the pathogenesis of KOA in the South Indian Hyderabad Population. The global population studies provide more reliable data and conclusions. Functional (i*n vitro*) studies are also recommended to elucidate the effects of the KOA polymorphisms and its pathogenicity and predisposition.

Author's contribution

PS has carried out the research experiment and manuscript preparation; SK is a clinician and has helped us with the cases and controls; KIA has helped in improving the manuscript; GS is a Co–I of the project and analysed the data; and HQ is the PI of the project, designed the study, analysed the statistical analysis data, edited and finalized the manuscript.

Conflicts of interest

There is no conflict of Interest towards this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jor.2019.05.001.

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