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Original Article

Genetic influence of growth and differentiation factor 5 gene polymorphism (+104T/C) on the development of knee osteoarthritis and its association with disease severity

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Abstract

Objective: The growth and differentiation factor 5 (GDF5) gene is recognized for its role in the development, repair, and maintenance of cartilage and bone. The present case-control study was conducted to detect the genetic association between GDF5 (+104T/C) single-nucleotide polymorphism (SNP) and primary knee osteoarthritis (KOA), as well as the possible association of SNP with the severity of KOA.

Material and Methods: The study included 50 patients with primary KOA and 50 healthy control subjects. The severity of the disease was assessed by using the Kellgren-Laurence (K-L) grading system and aided by the Western Ontario & McMaster Universities Osteoarthritis Index (WOMAC) score, visual analog scale (VAS) score, and tenderness score. The genetic association of the SNP with primary KOA was assessed by means of the TaqMan® allelic discrimination technique.

Results: The radiological assessment of patients according to the K-L grading system revealed a statistically significant association between the wild-type (TT) genotype and disease severity in both the right and left knees (p=0.049). The frequency distribution of patients with VAS score ≤6 was significantly higher in patients carrying the TT genotype (p=0.005) as compared to the CT and CC genotypes. The mean WOMAC score was significantly higher in patients carrying the TT genotype as compared to patients carrying the CC and CT genotypes (p=0.017). No statistically significant association was detected on comparing the frequency distribution of allele and genotype frequencies of the SNP in patients and healthy controls.

Conclusion: The results of the current study revealed a possible genetic association between GDF5 (+104T/C) SNP and the severity of KOA, which might be of benefit for the detection of patients with a high risk for disease progression. The present study did not detect an association between the SNP and development of KOA.

Keywords: Knee osteoarthritis, GDF5 polymorphism, allelic discrimination, K-L grading system, WOMAC Index Score

Introduction

Osteoarthritis (OA) is a complex chronic multifactorial age-associated disease, which has a profound effect on the functioning of synovial joints, primarily the knee, hip, and hands. The pathogenesis of the disease involves the irreversible destruction of the articular cartilage escorted by alterations in the homeostatic balance of chondrocyte cells and changes in other joint tissues (1).

Although the etiology and the exact pathogenesis of OA are not fully understood, the disease is a chronic degenerative condition of mobile joints, which might be associated with aging, hormonal, environmental, and genetic factors-major factors associated with its development and onset (2, 3).

The primary reason for seeking medical care following clinically diagnosing OA is the pain and loss of articular function (4). The prevalence and incidence of OA will continue to rise in the next few decades due to the rise in the overall lifespan and increase in obesity levels (5).

Research has demonstrated that genetic factors contribute enormously in the etiology and pathogenesis of OA. Growth and differentiation factor 5 (GDF5), which is also known as cartilage-derived morphogen protein of the transforming growth factor-β (TGF-β) super family, was shown to play a substantial role in the process of development, maintenance, and repair of cartilage and bone (6). Polymorphisms in the GDF5 gene have been involved in several skeletal developmental disorders, such as different forms of chondrodysplasia, symphalangism, and brachydactyly type C (7).

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The variations in genetic susceptibility in different ethnic groups has led us to attempt to examine the association between GDF5 gene polymorphism (rs143383) and knee osteoarthritis (KOA) in Beni Suef governorate in Upper Egypt. Further, attempts were made to detect an association between polymorphism and disease severity.

Material and Methods

Patients

The current case-control study included 50 primary KOA patients and 50 age-and-sex-matched healthy control subjects. The KOA patients (41 females and 9 males) were recruited from the outpatients' clinic of the Rheumatology and Rehabilitation department of Beni-Suef University Hospital, Beni Suef governorate, Egypt, between January 2015 and June 2015. The patients were diagnosed by a rheumatologist according to the American College of Rheumatology (ACR) classification for KOA (8). The patients with a history of knee or hip surgery, secondary OA, other rheumatic diseases, and knee trauma were excluded from the study. The healthy control subjects were unrelated Egyptian individuals with no family history of OA or other rheumatic diseases. The control group lived in the same geographical region as the patients. The patients and healthy control subjects included in the study were informed of the purpose of the study, and their consents were obtained.

Clinical assessment

The patients included in the study were subjected to full history taking, thorough clinical examination, and radiological investigations of both the knees. The patients were weighed with a calibrated beam balance to the nearest 0.1 kg, wearing the least possible clothes. The patients' standing height was measured in centimeters using a stadiometer. The Quetelet index was used to record the body mass index (BMI). The severity of disease reflected on radiographs was detected using the Kellgren-Laurence (K-L) grading system (9). This severity was categorized as follows: mild (K-L grade 2), moderate (K-L grade 3), and severe (K-L grade 4). The severity of KOA symptoms was assessed using the Western Ontario & McMaster Universities Osteoarthritis Index (WOMAC) (10). The intensity of pain was assessed by the 100-mm visual analog scale (VAS) (11). The patients were categorized into 2 groups: the first group represented patients with a VAS score ≤6, while the second group represented patients with a VAS score >6 (3). Tenderness was assessed by the tenderness score (TS) (12).

Genotyping

Approximately 2-3 mL of venous blood was collected by a sterile venipuncture using a sterile EDTA vacutainer. Genomic DNA was extracted from EDTA anti-coagulated whole blood using QIAamp DNA Mini Kit (QIAGEN; Hilden, Germany) according to the manufacturer's protocol.

The region of the GDF5 (+104T/C; rs143383) encompassing single-nucleotide polymorphism (SNP) was amplified using the TaqMan® 5′ allelic discrimination technique for amplifying and detecting specific polymorphisms in purified genomic DNA samples. The sense sequence was (5-AGCACACAGGCAGCATTACG-3), while the antisense sequence was (5-CCAGTCCCATAGTGGAAATG-3). The TaqMan® MGB probes/extension primers were (VIC AACTCGTTCTTGAAAGGAGAAAGCC) to detect the allele 1 sequence and (6FAM ACCGCCCCCTTTCTCCTGCACAACT) to detect the allele 2 sequence. The total PCR reaction volume was 20 µL: 40 ng/uL gDNA, 10 µL 2× Universal TaqMan master mix II (Applied Biosystems; Massachusetts, USA), and 0.5 µL 20× SNP assay mix, which was adjusted to a final volume of 20 µL using nuclease-free water.

After gDNA extraction was performed, the gDNA concentration was measured by means of NanoDrop® spectrophotometry (NanoDrop; Berlin, Germany).

The PCR was performed using a StepOne™ system (Applied Biosystems; Massachusetts, USA) under the following conditions: initial enzyme activation at 95°C for 10 min, followed by 40 cycles of amplification; denaturation at 95°C for 15 s; and annealing/extension for 1 min at 60°C. Florescence data collection was performed at the annealing/extension step for 6FAM and VIC dye.

Statistical analysis

The collected data was reviewed, and coding and statistical analysis was done by using the Statistical Package for the Social Sciences program (SPSS Inc.; Chicago, IL, USA) version 16 for Microsoft Windows. Mean, median, range, and standard deviation were calculated to measure the central tendency and dispersion of quantitative data, while the frequency of occurrence was calculated to measure the qualitative data. Student's t-test was used to determine the significance in the difference between two means. The chi-square (c^2) test was performed for comparing the qualitative data, and Fisher's exact test was used instead when the cell count was less than 5 to test the association between the genotype and primary KOA. The analysis of variance (ANOVA) test was used to determine the difference between more than two means. Multiple linear regression analysis was done to neutralize the effect of confounding factors on the WOMAC score. Genotype distributions were compared with those expected for samples from populations in the Hardy-Weinberg equilibrium (HWE) using a χ^2 test (1df). The level of significance was taken at $p<0.05$.

Results

In the current case-control study, the percentage of female patients was 82%, while the percentage of male patients was 18%. The mean age of patients was 56.52±8.09 years, while the mean age of the healthy control group was 54.85±8.79 years. The clinical and demographic data of patients and healthy control subjects are summarized in (Table 1).

Table 1. Demographic and radiological data of patients and controls

Variable	Cases N=50 (%)	Controls N=50 (%)	p
Age (years)			
Mean±SD	56.52±8.09	54.85±8.79	0.356
Sex			
Male:Female	9/41	9/41	
Disease duration (years)			
Mean±SD	7.04±4.40		
BMI (Kg/m^2)			
Mean±SD	34.78±5.70	28.61±3.61	<0.0001*
K-L score			
Grades (2/3/4)	20/19/11**		

BMI: Body Mass Index; kg/m^2: kilogram per meter squared; SD: standard deviation; K-L: Kellgren-Lawrence; N: number; %: percentage
*Significance difference ($p<0.05$); **20 patients with K-L grade 2, 19 patients with K-L grade 3, and 11 patients with K-L grade 4

Table 2. Association between GDF5 (rs143383) gene polymorphism and knee osteoarthritis in all study group controls, and in males and females (patients and controls)

	All study group			Females			Males		
	Cases N=50 (%)	controls N=50 (%)	X^2 and p	Cases N=41 (%)	controls N=41 (%)	X^2 and p	Cases (9) N=9 (%)	Controls (9) N=9 (%)	X^2 and p
Genotype				Genotype					
TT	20 (40.0)	12 (24.0)		15 (36.59)	10 (24.39)		5 (55.56)	2 (22.22)	
CC	14 (28.0)	13 (26.0)	X^2=4.01 p=0.134	11 (26.83)	11 (26.83)	X^2=1.71 p= 0.425	3 (33.33)	2 (22.22)	X^2= 4.15 p= 0.125
CT	16 (32.0)	25 (50.0)		15 (36.59)	20 (48.78)		1 (11.11)	5 (55.56)	
Allele[6]									
T	56 (56.0)	49 (49.0)	X^2=0.72	45 (54.88)	40 (48.78)	X^2=0.39	11 (61.11)	9 (50.0)	X^2=0.11
C	44 (44.0)	51 (51.0)	p=0.369	37 (45.12)	42 (51.22)	p=0.532	7 (38.89)	9 (50.0)	p=0.745

χ^2: chi-square; N: number; %: percentage -Significance difference (p<0.05); [6]Allele frequency was calculated according to Hardy-Weinberg equilibrium (HWE)

Table 3. Association of GDF5 (rs143383) gene polymorphism with clinical scores

					Range		
Variable		N	Mean	SD	Min	Max	p
Score	Genotype						
	CC	14	2.29	0.73	1.00	3.00	0.012*
Right Knee TS	TT	20	2.80	0.41	2.00	3.00	
	CT	16	2.13	0.89	1.00	3.00	
	CC	14	2.14	1.03	1.00	3.00	0.541
Left Knee TS	TT	20	2.40	0.75	1.00	3.00	
	CT	16	2.13	0.72	1.00	3.00	
	CC	14	3.36	0.74	2.00	4.00	0.049*
Right Knee K-L	TT	20	3.45	0.83	2.00	4.00	
	CT	16	2.81	0.83	2.00	4.00	
	CC	14	3.21	0.89	2.00	4.00	0.049*
Left Knee K-L	TT	20	3.45	0.83	2.00	4.00	
	CT	16	2.75	0.77	2.00	4.00	
	CC	14	65.87	15.36	41.60	92.70	0.017*
WOMAC	TT	20	78.51	12.22	41.60	79.20	
	CT	16	67.23	14.40	45.80	87.50	

K-L: Kellgren-Lawrence; WOMAC: Western Ontario & McMaster Universities Osteoarthritis Index; N: number; SD: standard deviation *Significant difference (p<0.05); TS: tenderness score

The frequency distribution of GDF5 (+104T/C; rs143383) SNP genotypes in primary KOA patients showed deviation from HWE (p=0.013), while the distribution of genotype frequencies in the healthy control subjects conformed to HWE (p=0.997).

Genotype and allele frequency distribution of GDF5 (+104T/C) in patients and healthy control subjects did not reveal any statistically significant differences between the two groups. Similarly, on categorizing both patients and control subjects according to gender, there was no statistically significant difference between males and females regarding the frequency distribution of allele and genotype frequencies of GDF5 (+104T/C) SNP (Table 2).

Next, we analyzed the genetic influence of GDF5 (+104T/C) SNP on the severity of primary KOA by comparing the frequency distribution of allele and genotype frequencies of the SNP in the TS, WOMAC score, K-L score, and VAS score for pain (Table 3).

The analysis of the association of the knee TS and the degree of tenderness in both the right and left knees in our study group revealed that the mean TS in the right knee was significantly higher in patients carrying the TT genotype as compared to patients carrying the heterozygous (TC) genotype and the variant (CC) genotype (p=0.012). Further, on analysis of the genetic association of GDF5 (+104T/C) SNP and radiological severity of primary KOA in the patient, according to the K-L grading scale, we detected that patients carrying the TT genotype had the highest mean K-L score in both the right and left knees as compared to patients carrying the CC and CT genotypes. The difference between the groups was statistically significant (p=0.049 and p=0.049, respectively) (Table 3).

Moreover, the analysis of the association between the WOMAC score and GDF5 gene SNP revealed that the patients carrying the TT gene had a significantly higher WOMAC score (p=0.017) as compared to patients carrying the CC and TT genotypes (Table 3). Moreover, a multiple linear regression analysis was performed to neutralize the effects of confounding factors, namely, BMI, age, and disease duration on the WOMAC score. The analysis revealed that after adjusting for BMI, age, and disease duration, the TT genotype of GDF5 (+104T/C) SNP can still be used to predict the WOMAC score (p<0.001) (Table 4).

Finally, we analyzed the association between the intensity of pain in our patients represented by the VAS score and GDF5 (+104T/C) SNP. Our results revealed that the frequency of patients carrying the TT genotype was the highest in patients whose VAS scores were <6 as compared to patients whose VAS scores were ≤6. On the other hand, the frequency of patients carrying the CT genotype was the highest among patients with pain intensity ≤6 as compared to patients with VAS scores >6. The frequency of patients carrying the CC

Table 4. Multiple linear regression analysis of confounding factors affecting the WOMAC score

Variable	Regression Coefficient	Standard Error	t^	95% CI of Regression Coefficient	p	$R^{2\delta}$
Genotype						
TT/CC	16.139	3.859	4.183	8.25-24.03	<0.001*	
BMI	1.237	0.417	2.967	0.38-2.09	0.006*	0.594
Disease	0.944	0.520	1.816	0.12-2.01	0.08*	
Duration						
Age	0.0646	0.245	2.635	0.14-1.147	0.013*	
Genotype						
TT/CT	16.603	3.594	4.619	9.27-23.93	<0.001*	0.505
BMI	0.610	0.303	2.014	-0.01-1.84	0.053	
Disease	0.933	0.444	2.100	0.03-1.84	0.044*	
Duration						
Age	0.685	0.232	2.954	0.21-1.16	0.006*	

BMI: Body Mass Index; CI: confidence interval; t^: statistic test used for null hypothesis showing that there is no linear relationship between the independent variables (genotype, BMI, age, and disease duration) and the dependent variable (WOMAC score); $^{\delta}R^2$: goodness-of-fit measure of a linear model *Significance level ($p<0.05$)

Table 5. Association of GDF5 (+104T/C) genotypes with VAS Score

	Genotype				
	CC	TT	CT		
VAS Score	N=14 (%)	N=20 (%)	N=16 (%)	X^2 and p	
VAS ≤6	6 (42.9%)	3 (15.0%)	11 (68.8%)	X^2=10.77	p=0.005*
VAS >6	8 (57.1%)	17 (85.0%)	5 (31.2%)		

VAS: visual analog scale; N: number; %: percentage; χ^2: chi-square *Significance level ($p<0.05$)

genotype with VAS scores ≤6 was higher than patients with VAS scores >6. The difference between the groups was statistically significant (p=0.005) (Table 5).

Discussion

Several studies have delineated the association of diverse genetic variants with the risk of OA (13-15). GDF5 (+104T/C), located in the 5′ untranslated region (5′-UTR) of the GDF5 gene, was one of the genetic variants that has garnered extensive attention as a genetic variant associated with OA. GDF5 (also known as cartilage-derived morphogenic protein 1, CDMP1) is one of the members of the TGF-β superfamily, which participates in the process development, repair, and maintenance of cartilage, bone, and soft tissues of the synovial joint (6). The GDF5 gene was found to be expressed in the articular cartilage of human adults and is involved in the processes of development, homeostasis, and repair of cartilage, bone, and articular cartilage (16).

The current case-control study revealed the lack of genetic influence of GDF5 (+104T/C) on the development of primary KOA. Our results were consistent with the results of the study performed by Tsuzou et al. (17), which conformed to the heterogeneous nature of OA genetic susceptibility. Tsuzou et al. (17) indicated that there were no statistically significant differences in the genotype and allele frequencies of the +104T/C SNP of the GDF5 gene in patients and controls (p>0.05). In addition, on categorizing the cases based on gender, no allele or genotype differences were found in the frequency distribution based on gender. Therefore, the results of the study performed by Tsezou et al. (17) indicated that GDF5 (+104T/C) SNP is not a risk factor for KOA in Greek Caucasians. Similarly, our results were in accordance with the results of the study conducted by Southam et al. (6), which revealed the absence of significant differences in genotype and allele frequency distribution of SNP in cases and healthy control subjects (p>0.05 and p=0.436, respectively). Similarly, these results were consistent with the results of the studies performed on the Korean population by Shin et al. (15) and Cao et al. (18).

However, the results of the current study were inconsistent with the results of the studies performed by Tawonsawtruk et al. (3) on the Thai population and the study performed by Mishara et al. (19) on the North Indian population, which revealed an association between rs143383 and the risk of KOA.

Generally, the GDF5 (+104T/C) SNP was found to be associated with the risk of developing human OA and degenerative disc diseases (20). However, the results of the current study regarding the lack of association between GDF5 (+104T/C) SNP and the susceptibility to KOA might imply that other polymorphic loci in the GDF5 gene might play a role in the susceptibility to KOA. Egli et al. (21) reported the detection of a second GDF5 polymorphism in the 5′-UTR region (rs143384) that could modulate the expression of GDF5 rs143383. Further, they identified a new polymorphism 2250ct that could have an influence on the GDF5 allelic expression, independent of rs143383. The effect of 2250ct on the allelic expression of GDF5 was corresponding to the effect of rs143383, i.e., a steady but moderate relative reduction in the expression of the order of 20%-25% (21).

Furthermore, the discrepancy between the results of the current study and previous studies conducted worldwide might be attributable to several factors. Reynard et al. (22) pointed out that the genetic effect of the rs143383 SNP was under the regulation of the epigenome at the DNA methylation level. They indicated that DNA methylation modulates the rate of the GDF5 expression in human cell lines and hypomethylation of the GDF5 locus is associated with augmentation in rs143383 allele imbalance. Moreover, Sub1, SP1, and SPP3 and DEAF trans-activating proteins bind two alleles of rs143383 and repress their transcription (23), resulting in the allelic imbalance between the rs143383 alleles. Therefore, methylation regulates GDF5 expression in cartilage and regulates the functional effect of rs143383 by altering the binding of SP1 and SPP3 and DEAF transcriptional repressors (5). In addition, differences in the levels of methylation in different joint tissues could result in differences in the odds ratio (OR) in different pathologies associated with GDF5 (+104T/C) SNP. In the meta-analysis conducted by Zhang et al. (2), it was revealed that the test of heterogeneity for rs143383 conducted on different populations was significant, proposing potential genetic heterogeneity between different populations. Taken together, ethnic differences in GDF5 methylation due to differences in environmental and other associated genetic factors might be the cause of such disparity in the results of different studies performed on

GDF5 (+104T/C) SNP in different populations (5). Finally, differences in genotyping methods and differences in patient's inclusion criteria might be other factors yielding inconsistent results in different studies.

Osteoarthritis diagnosis is based on both clinical and radiological disease assessments and X-ray is considered to be the gold standard used to confirm the diagnosis and is the main standard used in OA disease grading (24, 25). The grading of OA is based on the radiological classification of the K-L score and is usually aided by other general or specific joint clinical and functional scores (26-27).

In the present case-control study, radiological severity of KOA based on the K-L grading system revealed that the patients carrying the TT genotype had the highest K-L scores in both the right and left knees when compared to patients carrying the CC and CT genotypes (p=0.049).

Furthermore, we analyzed the WOMAC score, VAS score, and TS to detect the genetic influence of GDF5 (+104T/C) SNP on the degree of severity of primary KOA. Regarding the WOMAC score, the patients carrying the TT genotype had the highest mean WOMAC score when compared to patients carrying the CC and CT genotypes (p=0.017). Similarly, the frequency of patients with VAS scores >6 was higher among patients carrying the TT genotype when compared to patients carrying the CT and GG genotypes (p=0.005).

Our results regarding the association between GDF5 (+104T/C) SNP and the severity of KOA were consistent with the results of the study conducted by Valdes et al. (28) that reported a significant association between the T allele and tibiofemoral K-L grade and rs143383 SNP (p=0.0011). Our results were also consistent with the results of the study conducted by Minafra et al. (26) that indicated the presence of an association between the presence of the TT genotype and radiological severity of KOA represented by the K-L score (p=0.02).

The association between rs143383 and the progression of disease severity could be attributed to the results revealed in the original study conducted by Miayamoto et al. (16) that pointed out the influence of T allele on the transcription of the GDF5 gene with the resultant reduction in the transcriptional activity. This might imply that rs143383 SNP might influence the biological processes that are involved in joint damage that might be caused by its relation with the progression of the disease (16, 28).

The results of the current study revealed a possible genetic association between GDF5 (+104T/C) SNP and severity of KOA, which might facilitate the detection of patients with high risk for disease progression. The present study did not detect an association between the SNP and KOA development in Egyptian Caucasians in the Beni Suef governorate. Nevertheless, the current study should be regarded as hypothesis testing with its limitations. Further longitudinal studies using a larger number of patients are needed to define the association of GDF5 (+104T/C) SNP and the severity of KOA.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Beni-Suef University School of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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