Putative functional variants of PI3K/AKT/mTOR pathway are associated with knee osteoarthritis susceptibility

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Funding information
the guiding project of Health and Family Planning Commission of Changzhou, Grant/Award Number: No.wz201514; National Natural Science Foundation of China, Grant/Award Number: No. 81272017

Abstract

Background: Osteoarthritis (OA) is a degenerative musculoskeletal disease which causes joint deformity and pain and finally leads to limb dysfunction. Knee osteoarthritis (KOA) has the highest incidence among all kinds of OA. Strong evidence leads to the understanding that PI3K/AKT/mTOR signaling is very important in cartilage degeneration.

Methods: This research sought to understand the association between genetic variation of PI3K/AKT/mTOR genes and KOA susceptibility among Chinese population. All the genetic variants of PI3K/AKT/mTOR pathway were graded and selected using RegulomeDB database, and then, an association study including 278 osteoarthritis patients and 289 controls was conducted.

Results: Finally, eight SNPs' genotypes' distributions and susceptibility to KOA were presented. AKT1 rs2498789 was associated with KOA susceptibility in dominant genetic model (AA + GA vs GG) after adjusted for BMI, age, and gender: OR = 1.46, 95% CI: 1.03-2.05, P = .03. PIK3CA rs7646409 was also associated with KOA susceptibility (TC vs TT) after adjusted for BMI, age, and gender: OR = 0.58, 95% CI: 0.36-0.93, P = .02. PIK3CA rs7646409 (TC vs TT) with KOA risk was more significant in age < 60 group (P for heterogeneity was .03). Risk score showed significant association with KOA susceptibility after cumulative analysis (OR = 2.45, 95% CI: 1.35-4.45, P = .003).

Conclusions: This study shows that genetic variation of PI3K/AKT/mTOR is associated with KOA susceptibility in Chinese Han population, indicating that PI3K/AKT/mTOR is very important in KOA pathogenesis.

Keywords
osteoarthritis, PI3K/AKT/mTOR, polymorphism, regulomeDB

Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BMI, body mass index; CIs, confidence intervals; ECM, extracellular matrix; HWE, Hardy-Weinberg equilibrium; KOA, knee osteoarthritis; MMP, matrix metalloproteinase; ORs, odd ratios; SNP, single nucleotide polymorphisms.

Kejie Wang and Minjie Chu are contributed equally to this work.

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INTRODUCTION

Osteoarthritis (OA) is a kind of musculoskeletal disease which influences the bone, synovial tissues, and articular cartilage with no obvious regional and racial difference.\(^1\) Over million people are affected by OA worldwide, and it is an important reason for long-term immortality in China.\(^2,3\) OA commonly affects various human joints, and knee joints are most vulnerable. Pathology characteristics of knee osteoarthritis (KOA) are cartilage degeneration, cartilage extracellular matrix (ECM) deterioration, subchondral bone sclerosis, and synovitis.\(^4,5\) The clinical manifestations of KOA are joint swelling, pain, malformation, and lose of motion.\(^6\) KOA brings huge burden to family and society all over the world.\(^7\) Thus, it is urgent to explore detail pathophysiology and molecular biology mechanism of OA for early diagnosis and treatment.

The etiology of osteoarthritis has not been fully understood. Risk factors such as obesity, genetics, family susceptibility, local biomechanics, age, and previous trauma may lead to OA occurrence and progression.\(^8\) As the sole cell type in joint cartilage, the imbalance between apoptosis and proliferation of chondrocytes is very important for OA.\(^9\) Therefore, keeping the homeostasis between apoptosis and proliferation of chondrocytes may facilitate cartilage repair and symptoms relieve of OA.\(^10\) P13K/AKT/mTOR pathway has received substantial attentions because it plays a crucial role during several characteristic alterations of cartilage such as expression of matrix metalloproteinase (MMP) or a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) which will finally lead to the apoptosis of chondrocytes.\(^10\) P13K/AKT/mTOR pathway belongs to serine/threonine protein kinase family. Inhibiting P13K/AKT/mTOR pathway could relieve inflammation response in rats with OA which means that P13K/AKT/mTOR pathway participates in the pathology of OA.\(^11\)

RegulomeDB, which is a database integrating data from Encyclopedia of DNA Elements project, illuminates regulatory variants’ function in human genes. Based on the functional confidence of variants, RegulomeDB provides a scoring system, which ranges from 1 to 6. A variant will possibly affect gene expression or transcription factor binding if its score is low. In summary, RegulomeDB is a powerful tool for genetic association study.

In this research, we assumed that P13K/AKT/mTOR genetic variants are potential pivotal targets for diagnosis or treatment of OA. To verify it, an association study was conducted between P13K/AKT/mTOR genetic variants identified by RegulomeDB and KOA susceptibility.

MATERIALS AND METHODS

In this research, patients were diagnosed with KOA according to American College of Rheumatology (ACR) classification of KOA from 2013 to 2017 at the Department of Orthopaedics, Changzhou First People’s Hospital, Jiangsu, China.\(^12\) Anteroposterior weight-bearing radiographs of every patient’s affected knees were taken. A group of orthopedist and radiologist evaluated the X-ray of knee to give a 0-4 score based on the Kellgren-Lawrence (KL) classification of KOA.\(^13\) Posttraumatic arthritis, postseptic arthritis, inflammatory arthritis (autoimmune disease, rheumatoid arthritis, or septic arthritis), developmental dysplasia, and other etiologies of knee were excluded. At the same hospital during the same period, age- and sex-matched healthy volunteers were recruited. All controls reported no history of KOA and other joint disease.

Two trained interviewers inquired each case and control subject to collect demographic information. Every participant written a consent, and then, 5 mL peripheral blood was taken. Each participant’s weight and height were measured accurate to 0.1 kg and 1 cm. Body mass index (BMI) was calculated. This research was agreed by Changzhou First People’s Hospital’s Human Research Ethics Committees.

This study tries to explore whether potential functional variations were associated with knee osteoarthritis of Chinese Han population. We select seven important genes (mTOR, ULK1, ULK2, ATG13, AKT1, AKT2, and PIK3CA) in P13K/AKT/mTOR pathway.\(^14,15\) The lower RegulomeDB score is, the more possible these variants will have functional significance. With this principle, 90 SNPs whose RegulomeDB scores range from 1 to 2b were selected. And also according to the information from UCSC database (GRCh37/hg19), linkage disequilibrium (LD) <0.8 and minor allele frequency (MAF) >0.05, 12 potentially functional genetic variants of P13K/AKT/mTOR were finally singled out.

Genomic DNA was extracted according to the method described before.\(^16\) Genotyping was performed with Sequenom’s MassARRAY® iPLEX assay following instructions of the manufacturer. Genotyping of three SNPs was failure because of probe design. Finally, 9 SNPs were genotyped successfully with over 95% call rate. We randomly selected more than 10% samples to test again for quality control with over 99% consistency. We used Student’s t tests and chi-square test to detect demographic data or genotypes’ distribution difference between different groups for continuous

| Table 1 | Distributions of select variables in OA cases and controls |
|---------|-----------------|-----------------|-----------------|
| Variables | Case (n = 278) | Control (n = 289) | P |
| Age, y (mean ± SD) | 62.00 ± 10.55 | 61.13 ± 10.92 | .34 |
| Gender | | | |
| Male | 82(29.5) | 87(30.1) | .87 |
| Female | 196(70.5) | 202(69.9) | |
| BMI | | | |
| BMI < 25 | 150(54.0) | 189(66.5) | .01 |
| BMI ≥ 25 | 128(46.0) | 95(33.5) | |
| KL classification | | | |
| 1-2 | 135 | | |
| 3-4 | 143 | | |

Note: Bold font presents P < .05.
| Gene | Genotype | Case | Control | OR (95% CI) | P  | OR (95% CI) | P  |
|------|----------|------|---------|-------------|----|-------------|----|
| ATG13 rs10838610 | AA      | 199 (72%) | 197 (68%) | 0.91 (0.63-1.31) | .6 | 0.94 (0.64-1.37) | .74 |
|        | AG      | 75 (27%) | 82 (28%) | 0.30 (0.08-1.1) | .07 | 0.30 (0.08-1.13) | .08 |
|        | GG      | 3 (1%)   | 10 (4%)  | 0.84 (0.59-1.2) | .34 | 0.87 (0.60-1.25) | .44 |
|        | GG vs.AA | 0.31 (0.08-1.12) | .07 | 0.31 (0.08-1.15) | .08 |
|        | ADD     | 0.79 (0.58-1.1) | .16 | 0.81 (0.59-1.13) | .22 |
|        | GG vs. AG vs. AA | 0.84 (0.52-1.37) | .49 | 0.75 (0.45-1.23) | .25 |
|        | GG vs. AG vs. AA | 0.87 (0.60-1.25) | .25 | 0.81 (0.56-1.13) | .25 |
| ULK1 rs1134574 | AA      | 243 (88%) | 247 (86%) | 0.79 (0.58-1.1) | .16 | 0.81 (0.59-1.13) | .22 |
|        | GA      | 34 (12%) | 41 (14%) | 0.79 (0.58-1.12) | .49 | 0.75 (0.45-1.23) | .25 |
|        | GG      | 0 (0%)   | 0 (0%)   | 0.84 (0.52-1.37) | .49 | 0.75 (0.45-1.23) | .25 |
|        | GG vs.AA | 0.75 (0.45-1.23) | .25 | 0.81 (0.56-1.13) | .25 |
|        | GG vs. GA vs. AA | 0.84 (0.52-1.37) | .49 | 0.75 (0.45-1.23) | .25 |
| ULK2 rs157389 | TT      | 168 (61%) | 163 (56%) | 1.03 (0.5-2.11) | .93 | 0.89 (0.42-1.87) | .76 |
|        | TC      | 89 (33%) | 110 (38%) | 0.79 (0.55-1.12) | .18 | 0.81 (0.56-1.16) | .25 |
|        | CC      | 17 (6%)  | 16 (6%)  | 1.30 (1.0-2.14) | .24 | 0.82 (0.58-1.16) | .26 |
|        | CC vs. TC vs. TT | 0.82 (0.58-1.16) | .24 | 0.82 (0.58-1.16) | .26 |
|        | CC vs. TC vs. TT | 0.84 (0.52-1.37) | .49 | 0.75 (0.45-1.23) | .25 |
|        | ADD     | 0.84 (0.52-1.37) | .49 | 0.75 (0.45-1.23) | .25 |
| AKTI rs2498789 | GG      | 119 (44%) | 148 (52%) | 1.48 (1.04-2.11) | .03 | 1.58 (1.10-2.28) | .01 |
|        | GA      | 126 (46%) | 106 (37%) | 1.08 (0.60-1.92) | .08 | 1.07 (0.59-1.91) | .37 |
|        | AA      | 26 (10%) | 30 (11%) | 0.90 (0.52-1.56) | .70 | 0.87 (0.49-1.52) | .62 |
|        | AA vs. GA vs. GG | 0.90 (0.52-1.56) | .70 | 0.87 (0.49-1.52) | .62 |
|        | ADD     | 1.18 (0.92-1.52) | .2 | 1.20 (0.93-1.55) | .16 |
| AKTI rs3001371 | TT      | 106 (39%) | 117 (41%) | 1.33 (0.79-2.25) | .29 | 1.39 (0.81-2.39) | .23 |
|        | CT      | 127 (46%) | 137 (47%) | 1.33 (0.79-2.25) | .29 | 1.39 (0.81-2.39) | .23 |
|        | CC      | 41 (15%) | 34 (12%) | 0.98 (0.52-1.56) | .70 | 0.87 (0.49-1.52) | .62 |
|        | CC vs. CT vs. TT | 0.98 (0.49-1.52) | .70 | 0.87 (0.49-1.52) | .62 |
|        | ADD     | 1.12 (0.88-1.43) | .37 | 1.13 (0.88-1.45) | .34 |
| AKTI2 rs3730051 | TT      | 134 (49%) | 132 (46%) | 0.93 (0.65-1.32) | .68 | 1.02 (0.71-1.46) | .91 |
|        | TC      | 114 (41%) | 121 (42%) | 0.79 (0.45-1.37) | .04 | 0.86 (0.49-1.52) | .61 |
|        | CC      | 28 (10%) | 35 (12%) | 0.9 (0.7-1.15) | .41 | 0.96 (0.74-1.23) | .74 |
|        | CC vs. TC vs. TT | 0.9 (0.7-1.15) | .41 | 0.96 (0.74-1.23) | .74 |
|        | ADD     | 0.90 (0.64-1.25) | .52 | 0.98 (0.70-1.38) | .93 |
|        | TT vs. CT vs. TT | 0.82 (0.48-1.38) | .45 | 0.85 (0.50-1.47) | .57 |
|        | ADD     | 0.9 (0.64-1.25) | .52 | 0.98 (0.70-1.38) | .93 |
|        | TT vs. CT vs. TT | 0.82 (0.48-1.38) | .45 | 0.85 (0.50-1.47) | .57 |
|        | ADD     | 0.9 (0.64-1.25) | .52 | 0.98 (0.70-1.38) | .93 |
| AKTI2 rs7247515 | CC      | 247 (90%) | 258 (89%) | 0.91 (0.53-1.57) | .73 | 0.96 (0.55-1.68) | .9 |
|        | CT      | 27 (10%) | 31 (11%) | 0.91 (0.53-1.57) | .73 | 0.96 (0.55-1.68) | .9 |
|        | TT      | 1 (0%) | 0 (0%) | 0.94 (0.55-1.62) | .83 | 0.99 (0.57-1.72) | .98 |
|        | TT vs. CC | 0.94 (0.55-1.62) | .83 | 0.99 (0.57-1.72) | .98 |
|        | ADD     | 0.98 (0.58-1.66) | .95 | 1.02 (0.6-1.75) | .93 |
and categorical variables. Hardy-Weinberg equilibrium (HWE) was calculated for each SNP in controls. Logistic regression was adopted to estimate 95% confidence intervals (CIs) or odd ratios (ORs) as an evaluation of association with the KOA susceptibility, adjusted for BMI, gender, and age. Corresponding subgroups’ heterogeneity was detected with chi-square-based Q test. Cumulative effects of all genotyped SNPs were also evaluated with a risk score analysis using a linear of genotypes (coded as 0, 1, and 2). SPSS Statistics Version 18.0 software was used to finish above calculation.

3 | RESULTS

The clinical variable distribution between controls and cases is demonstrated in Table 1. Gender and age of two groups were comparable (P > .05). All of them have over 95% for success rates of genotyping. One variant rs892119 was not in accordance with HWE. Genotypes’ distributions and susceptibility to KOA of the remaining eight SNPs are presented in Table 2.

**TABLE 2 (Continued)**

| Gene     | Genotype | Case | Control | OR (95% CI) | P  | OR (95% CI)a | P  |
|----------|----------|------|---------|-------------|----|--------------|----|
| PIK3CA   | rs7646409| TT   | 237 (87%) | 225 (81%)  | .063 (0.39-1.00) | .05 | 0.58 (0.36-0.93) | .02 |
|          |          | TC   | 35 (13%)  | 53 (19%)   | .63 (0.39-1.00) | .05 | 0.58 (0.36-0.93) | .02 |
|          |          | CC   | 0 (0%)    | 0 (0%)     | .63 (0.39-1.00) | .05 | 0.58 (0.36-0.93) | .02 |
|          |          | CC + TC vs. TT |          | 0.63 (0.39-1.00) | .05 | 0.58 (0.36-0.93) | .02 |
|          |          | ADD  |          |            | .63 (0.39-1.00) | .05 | 0.58 (0.36-0.93) | .02 |

Notes: Bold font presents P < .05.
aAdjusted for age, gender, and BMI.

AKT1 rs2498789 shown borderline association with KOA susceptibility in dominant genetic model (AA + GA vs GG): OR = 1.39, 95% CI: 1.00-1.94, P = .05. The association remained after adjusted for BMI, age, and gender in dominant model: OR = 1.46, 95% CI: 1.03-2.05, P = .03. PIK3CA rs7646409 also shown borderline association with increased risk of KOA susceptibility (TC vs TT): OR = 0.63, 95% CI: 0.39-1.00, P = .05. After adjusted for BMI, gender, and age, the results were more significant: OR = 0.58, 95% CI: 0.36-0.93, P = .02.

Stratified analyses (Table 3) were preceded in positive results mentioned above. Stratified analyses showed that PIK3CA rs7646409 (TC vs TT) with KOA susceptibility was more obvious in age <60 group (P for heterogeneity test was .03). A risk score analysis was used to calculated cumulative effects of the remaining 8 genetic variants (Table 4). The mean cumulative risk score of controls (~0.03 ± 0.28) was lower than that of cases (~0.11 ± 0.31) (P for T test <.05). Logistic regression analyses indicated that cumulative risk score was associated with KOA susceptibility (Table 4).

**TABLE 3 Stratified analyses of rs2498789 and rs7646409 genotypes associated with patients of KOA by selected variables**

| rs2498789AA + GA vs. GG | rs7646409TC vs. TT |
|-------------------------|--------------------|
| Case | Control | OR (95% CI)a | P  | Phet | Case | Control | OR (95% CI)a | P  | Phet |
| Age | | | | | | | | | |
| Age < 60 | 62/49 | 69/69 | 1.38 (0.82-2.32) | .23 | .77 | 11/100 | 30/107 | 0.31 (0.14-0.69) | .00 | .03 |
| Age ≥ 60 | 90/70 | 67/79 | 1.53 (0.96-2.43) | .07 | .37 | 24/137 | 23/118 | 0.95 (0.50-1.78) | .87 | .55 |
| Gender | | | | | | | | | |
| Male | 43/36 | 39/48 | 1.59 (0.84-2.99) | .15 | .74 | 7/72 | 16/70 | 0.45 (0.17-1.17) | .10 | .55 |
| Female | 109/83 | 97/100 | 1.40 (0.93-2.11) | .11 | .45 | 28/165 | 37/155 | 0.63 (0.36-1.09) | .10 | .55 |
| BMI | | | | | | | | | |
| BMI < 25 | 88/59 | 88/97 | 1.67 (1.08-2.60) | .02 | .29 | 18/130 | 33/149 | 0.62 (0.33-1.16) | .14 | .75 |
| BMI ≥ 25 | 64/60 | 45/49 | 1.15 (0.67-1.97) | .62 | .29 | 17/107 | 20/71 | 0.53 (0.26-1.11) | .09 | .55 |
| KL | | | | | | | | | |
| KL12 | 76/52 | 136/148 | 1.59 (1.03-2.45) | .03 | .58 | 21/112 | 53/225 | 0.76 (0.43-1.33) | .33 | .17 |
| KL34 | 74/65 | 136/148 | 1.34 (0.87-2.05) | .18 | .37 | 13/122 | 53/225 | 0.41 (0.21-0.81) | .01 | .55 |

Note: Bold font presents P < .05.
aAdjusted for age, gender, and BMI.
bP value for heterogeneity test.
TABLE 4 Cumulative risk scores of eight SNPs on the risk of KOA

|       | Case    | Control | P  | OR (95% CI)       | P   |
|-------|---------|---------|----|-------------------|-----|
| Risk score | -0.11 ± 0.31 | -0.03 ± 0.28 | .03 | 2.45 (1.35-4.45) | .003 |

Note: Bold font presents P < .05.

P for t test.

Adjusted for age, gender, and BMI.

4 | DISCUSSION

In this article, an association study was conducted between putative functional variants of PI3K/AKT/mTOR pathway and KOA. Our results show that AKT1 rs2498789 and PIK3CA rs7646409 were associated with KOA susceptibility in Chinese Han population. Different age-group of PIK3CA rs7646409 showed heterogeneity in our research, which means gene-environment interaction exists between genetic variants of PIK3CA rs7646409 and age.

The PI3K/AKT/mTOR pathway is a pivotal axis in various cellular processes including apoptosis, metabolism, angiogenesis, transcription, and cell cycle. And it is found abnormal expressed in many cancers. Previous studies have indicated that PI3K/AKT/mTOR is very important during pathogenesis of OA. Inhibition of PI3K/AKT/mTOR increases articular chondrocyte autophagy and ameliorates the progression of osteoarthritis in rats. Other research reported that PI3K/AKT/mTOR pathway’s activation can promote chondrocyte autophagy and protect against cartilage injury. Progression of osteoarthritis is affected by inflammatory cytokines. PI3K/AKT/mTOR signaling pathway could mediate TNF-α mRNA expression and NF-κB activation in osteoblasts. Another research reported that inhibition of PI3K/AKT/mTOR could protect chondrocytes against inflammation in osteoarthritis. PI3K/AKT/mTOR activation is essential for the chondrocyte proliferation and protection of chondrocytes from apoptosis. Thus, the detail mechanism of PI3K/AKT/mTOR in OA remains unclear.

In this study, we found AKT1 rs2498789 was associated with KOA. AKT1 which involved in protein synthesis and cellular survival pathway is very important for muscle or skeletal hypertrophy and general tissue growth. AKT1 knockout mouse revealed spontaneous apoptosis and growth retardation in tissues such as thymus and testes. AKT1 has also been shown connection with kinds of cancer since it could block apoptosis and therefore increase cell survival. Beyond that AKT1 is an important signaling factor in insulin signaling pathway. The RegulomeDB score of AKT1 rs2498789 is 2b. ChIP-seq data showed that rs2498789 could influence IKZF1 expression. IKZF1 encodes Ikaros, which is a zinc-finger transcription factor related with cytokine expression maintenance of mature lymphocytes. Further researches need to be done to clarify how AKT1 participates in the pathology of OA.

However, there are several limitations in our study. First, the samples in our study are relatively small. Further independent studies with large sample sizes are necessary to confirm our findings. Second functional studies should be conducted to unravel the molecular mechanisms underlying this association. But it is very difficult to collect the cartilage from normal people due to limited donors. So it is feasible to compare degenerative cartilage and relative “normal” cartilage from patients who underwent total knee joint replacement in future. Third, our study finds the association in Chinese Han population, and multicenter association should be conducted between different races to confirm our findings in future.

5 | CONCLUSION

This research indicated that potential functional genetic variation in PI3K/AKT/mTOR pathway is associated with KOA susceptibility. Further functional studies are essential to illuminate the molecular pathology of KOA.

ACKNOWLEDGMENTS

This study was supported by the guiding project of Health and Family Planning Commission of Changzhou (No. w201514) and National Natural Science Foundation of China (No. 81272017).

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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How to cite this article: Wang K, Chu M, Wang F, Zhao Y, Chen H, Dai X. Putative functional variants of PI3K/AKT/mTOR pathway are associated with knee osteoarthritis susceptibility. J Clin Lab Anal. 2020;34:e22340. https://doi.org/10.1002/jcla.22340