CONCISE REPORT

Association of polymorphisms in the peroxisome proliferator-activated receptor γ gene and osteoarthritis of the knee

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Objectives: To study the association between two common polymorphisms in the peroxisome proliferator-activated receptor γ (*PPAR γ*) gene and susceptibility to, and severity of, osteoarthritis in a French-Canadian population.

Methods: Genomic DNA was obtained from 172 patients with osteoarthritis and 210 ethnically matched healthy controls. Genotyping for the polymorphisms in the *PPAR γ* gene (Pro12Ala and C1431T) was carried out using polymerase chain reaction–restriction fragment length polymorphism. The standard Kellgren–Lawrence grading score and the French version of the Western Ontario and McMaster Universities Osteoarthritis Index were used to assess the radiological and functional severity of the disease. Estimated haplotypes were generated using the expectation maximisation algorithm. Genotype and allele frequencies were analysed using the χ^2 test.

Results: Genotype and allele frequencies for either polymorphism in the *PPAR γ* gene did not differ significantly between patients with osteoarthritis and controls. Moreover, no significant differences were observed after stratification of patients according to age at disease onset, radiological or functional severity. Similarly, haplotype analysis of both polymorphisms in the *PPAR γ* gene showed no association of any haplotype with susceptibility to, or severity of, osteoarthritis.

Conclusion: These findings suggest that the examined polymorphisms in the *PPAR γ* gene do not contribute to susceptibility to, or severity of, osteoarthritis in the French-Canadian population.

Osteoarthritis is the most common form of arthritis and is a leading cause of disability. Although the aetiology and pathogenesis of osteoarthritis are unknown, epidemiological and genetic studies have shown that genetic factors are strong determinants in osteoarthritis.¹ Therefore, identifying susceptibility genes is a promising approach to understanding the molecular basis and the pathogenesis of osteoarthritis.

Emerging evidence indicates that peroxisome proliferator-activated receptor γ (*PPAR γ*) has protective effects in osteoarthritis. *PPAR γ* activation prevents the expression of several inflammatory genes responsible for the pathogenesis of osteoarthritis, including interleukin 1 β , inducible nitric acid synthase, cyclo-oxygenase-2 and microsomal prostaglandin E synthase-1.^{2–4} *PPAR γ* activation also seems to be chondroprotective by negatively regulating the expression of matrix metalloproteinase-1 and matrix metalloproteinase-13 and by preventing proteoglycan degradation.^{2,3,5,6} In

addition, treatment with a *PPAR γ* activator, pioglitazone, shows beneficial effects in an experimental model of osteoarthritis.⁷ These data suggest that *PPAR γ* could be a candidate gene for susceptibility to osteoarthritis. Several genetic variants in the *PPAR γ* gene have been described, the most prevalent being Pro12Ala and C1431T. The Pro12Ala polymorphism has been associated with improved insulin sensitivity, reduced risk of myocardial infarction and atherosclerosis.⁸ The C1431T polymorphism has been associated with increased plasma level of leptin, reduced risk of coronary artery disease and survival in immunoglobulin A (IgA) nephropathy.⁹

As *PPAR γ* has protective properties in osteoarthritis, we conducted a case–control study to evaluate whether the Pro12Ala and C161T polymorphisms could influence susceptibility to, or severity of, osteoarthritis in a French-Canadian population.

PARTICIPANTS AND METHODS

Participants

We recruited 172 patients with primary knee osteoarthritis from the outpatient rheumatology clinic at the Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada. All patients were diagnosed according to the criteria of the American College of Rheumatology.⁹ Patients with autoimmune diseases, patellofemoral osteoarthritis or osteoarthritis secondary to other conditions (including inflammation, sepsis, metabolic abnormalities and trauma) were excluded.

The 210 controls were recruited from outpatient clinics at the Centre Hospitalier de l'Université de Montréal. Eligible participants were unrelated people, aged ≥ 55 years, who had no history of arthritis, knee trauma or pain. All patients and controls were French-Canadians who lived in or around Montreal. The ethics committees of Notre-Dame Hospital approved the study and participants gave written informed consent.

For each patient, radiographs of the knees bearing anteroposterior weight were taken and graded on a 5-point scale (0–4) according to Kellgren and Lawrence.¹⁰ Radiographic findings were classified into mild (Kellgren–Lawrence grade 1 or 2) or severe (Kellgren–Lawrence grade 3 or 4). Patients were also evaluated for functional severity using the Western Ontario and McMaster University Osteoarthritis (WOMAC) Index,¹¹ a validated disease-specific questionnaire dealing with the severity of joint pain (5 questions), stiffness (2 questions) and physical functioning (17 questions). The Visual Analogue Scale (0, best; 100, worse) version of the index was used, with

Abbreviations: PPAR, peroxisome proliferator-activated receptor; WOMAC Index, Western Ontario and McMaster University Osteoarthritis Index

Table 1 Characteristics of the study participants

	Patients with knee osteoarthritis (n = 172)	Controls (n = 210)
Age (years)	63.9 (8.5)	63.6 (6.6)
Sex (male:female)	57:112	62:148
Body mass index (kg/m ²)	32.1 (5.9)	26.7 (4.2)
Age at onset (years)	55.5 (9.1)	
Disease duration (years)	8.5 (6.0)	
Kellgren–Lawrence grade		
1–2	39 (22.6%)	
3–4	133 (77.3%)	
WOMAC score		
0–104	83 (48.8%)	
105–240	86 (51.2%)	

WOMAC, Western Ontario and McMaster University Osteoarthritis Index.

Values are mean (SD) or n (%).

the patient assessing each question on a 100 mm visual analogue scale. The total WOMAC Index was calculated by the summation of all 24 components, with 2400 mm being the worst possible score.

Genotyping and analysis of polymorphisms in the PPAR γ gene

Genomic DNA was prepared from whole blood samples by using QIAmp DNA Blood Midi Kits (Qiagen, Mississauga, Ontario, Canada), according to the manufacturer's recommended protocol. The polymorphisms in the PPAR γ gene were analysed using polymerase chain reaction and restriction fragment length polymorphism as described previously.^{12–13} The following primers were used: Pro12Ala, sense 5'-GCCAATTCAAGCCAGTC-3' and antisense 5'-GATATGTTTGACAGAGTGTATCAGTGAAGGAATCGCTTTC-CG-3'; C1431T sense 5'-AGGTTTGCTGAATGTGAAGC-3' and antisense 5'-GGTGAAGACTCATGTCTGT-3'.

Statistical analysis

Data were analysed using SPSS V.13.0. The differences in genotype distribution and allele frequency among groups were analysed using the χ^2 test. Maximum-likelihood haplotype frequencies were calculated using an expectation-maximisation algorithm¹⁴ (http://www.istech.info/istech/board/login_form.jsp).

RESULTS

Frequencies of polymorphisms in the PPAR γ gene in patients with osteoarthritis and in controls

Table 1 shows the demographic and clinical characteristics of the study population. Genotype and allele frequencies of the Pro12Ala polymorphism did not differ markedly between patients with osteoarthritis and controls (table 2). We also found no major differences in the genotype and allele frequencies of the C1431T polymorphism between both populations (table 2). As the proportion of the homozygous participants was small (1.8% for Pro12Ala and 2.3% for C1431T), the heterozygous and homozygous variant genotypes were combined in further analyses.

PPAR γ gene polymorphism in patients with osteoarthritis stratified by age at disease onset, and radiological and functional severity

To determine the effect of polymorphisms in the PPAR γ gene on age at disease onset, patients were divided into two groups according to the median age at onset: early-onset patients (onset at <55.5 years) and late-onset patients (onset at $\geq$ 55.5 years), and PPAR γ genotype distribution and allele frequencies were compared. The genotype distribution and allele frequencies of the Pro12Ala and C1431T polymorphisms were not significantly different between patients with early-onset osteoarthritis, patients with late-onset osteoarthritis and controls (table 3).

We also examined the effect of PPAR γ polymorphisms on the risk for severe radiographic or severe functional osteoarthritis. Patients with severe radiographic osteoarthritis had Kellgren–Lawrence grade 3 or 4, whereas those with mild radiographic osteoarthritis had a Kellgren–Lawrence grade of 1 or 2. Patients with severe functional osteoarthritis had a WOMAC Index >104, whereas those with mild functional osteoarthritis had WOMAC index $\leq$ 104.

We found no major difference in the genotype distributions and allele frequencies of both polymorphisms between patients with severe or mild radiographic osteoarthritis, or severe or mild functional osteoarthritis, and controls.

Haplotype analysis

To investigate whether one particular haplotype is associated with susceptibility to osteoarthritis, haplotype frequencies of both single-nucleotide polymorphisms were estimated using the maximum-likelihood estimation method.¹⁴ The frequencies of the four possible haplotypes were not significantly different between patients with osteoarthritis and controls.

Table 2 PPAR γ genotype distributions and allelic frequencies in patients with osteoarthritis and in controls

Polymorphism	Genotype n (%) [*]		Allele n (%) [†]	
	Patients with osteoarthritis (n = 172)	Controls (n = 210)	Patients with osteoarthritis (n = 344)	Controls (n = 420)
Pro12Ala				
Pro12Pro	138 (80.2)	159 (75.7)	Pro307 (89.2)	366 (87.1)
Pro12Ala	31 (18)	48 (22.9)	Ala37 (10.7)	54 (12.8)
Ala12Ala	3 (1.8)	3 (1.4)		
χ^2	1.37		0.79	
p Value	0.50		0.37	
C1431T				
CC	135 (78.48)	163 (77.6)	C303 (88.1)	371 (88.3)
CT	33 (19.1)	44 (20.9)	T41 (11.9)	49 (11.6)
TT	4 (2.3)	3 (1.4)		
χ^2	0.57		0.01	
p Value	0.75		0.91	

PPAR γ , peroxisome proliferator-activated receptor γ .

^{*}Controls versus patients, χ^2 test, 3 $\times$ 2 contingency table.

[†]Controls versus patients, χ^2 test, 2 $\times$ 2 contingency table.

Table 3 Polymorphisms in the peroxisome proliferator-activated receptor γ gene in patients with osteoarthritis stratified by age at disease onset, and radiological and functional severity

Polymorphism	Feature of osteoarthritis (clinical subtypes)					
	Age at onset*		Radiographic osteoarthritis†		Functional osteoarthritis‡	
	Early (n = 83)	Late (n = 89)	Mild (n = 39)	Severe (n = 133)	Mild (n = 84)	Severe (n = 87)
Pro12Ala						
Genotype distribution						
Pro12Pro	66 (79.5%)	72 (80.9%)	31 (79.5%)	107 (80.5%)	67 (79.8%)	70 (80.5%)
Pro12Ala+Ala12Ala	17 (20.5%)	17 (19.1%)	8 (20.5%)	26 (19.5%)	17 (20.2%)	17 (19.5%)
χ^2	0.48	0.95	0.26	1.05	0.55	0.78
p Value	0.49	0.32	0.61	0.30	0.45	0.37
Allele frequencies						
Pro12	146 (88.0%)	161 (90.4%)	69 (88.5%)	238 (89.5%)	149 (88.7%)	156 (89.7%)
Ala12	20 (12.0%)	17 (9.6%)	9 (11.5%)	28 (10.5%)	19 (11.3%)	18 (10.3%)
χ^2	0.32	2	0.29	1.59	0.66	1.32
p Value	0.56	0.15	0.58	0.20	0.41	0.25
C1431T						
Genotype distribution						
CC	64 (77.1%)	71 (79.8%)	32 (82.1%)	103 (77.4%)	66 (78.6%)	68 (78.2%)
CT+TT	19 (22.9%)	18 (20.2%)	7 (17.9%)	30 (22.6%)	18 (21.4%)	19 (21.8%)
χ^2	0.06	0.06	0.24	0.05	0	0.004
p Value	0.80	0.81	0.62	0.82	1	0.94
Allele frequencies						
C	144 (86.7%)	159 (89.3%)	70 (89.7%)	233 (87.6%)	148 (88.1%)	153 (87.9%)
T	22 (13.2%)	19 (10.7%)	8 (10.3%)	33 (12.4%)	20 (11.9%)	21 (12.1%)
χ^2	0.28	0.12	0.13	0.08	0.007	0.01
p Value	0.59	0.72	0.72	0.77	0.93	0.89

The χ^2 test was used with the 2×2 contingency table.

*Patients with early-onset osteoarthritis were <55.5 years old at disease onset and those with late-onset disease were ≥55.5 years old.

†Patients with severe radiographic osteoarthritis had Kellgren–Lawrence grade 3 or 4 and those with mild radiographic osteoarthritis had Kellgren–Lawrence grade 1 or 2.

‡Patients with severe functional osteoarthritis had Western Ontario and McMaster University Osteoarthritis (WOMAC) Index >104 and those with functionally mild osteoarthritis had WOMAC Index ≤104.

We also found no differences in *PPAR* γ haplotype frequencies between controls and patients with osteoarthritis stratified according to age at onset, sex, and radiological and functional severity of osteoarthritis (data not shown).

DISCUSSION

This study is the first attempt to evaluate the role of two *PPAR* γ gene polymorphisms, Pro12Ala and C1431T, in the pathogenesis of osteoarthritis. We found no evidence of an association of these polymorphisms with the susceptibility to, or the severity of, osteoarthritis.

The observed lack of association between polymorphisms in the *PPAR* γ gene and susceptibility to osteoarthritis may simply reflect that these polymorphisms have a minor or no role in the susceptibility to osteoarthritis. Another possibility is that the polymorphisms in the *PPAR* γ gene may be responsible for the pathogenesis of osteoarthritis, but this influence was too small to be detected with our study size, and a larger sample size may be required. Alternatively, a possible association may have been weakened by disease heterogeneity, environmental factors or gene–environment interactions.

The polymorphisms in the *PPAR* γ gene may be associated with the severity of, rather than the susceptibility to, the disease. However, stratification based on severe radiological damage or functional disability showed no association with either single-nucleotide polymorphisms or haplotypes. The patients in our study had longstanding osteoarthritis (mean duration of about 8.5 years) and were all undergoing active management of their disease, which would tend to obscure a possible association. Furthermore, measures of disease severity at only a single point are unlikely to reflect the influence that a gene polymorphism may have on development of osteoarthritis. A prospective study with an inception cohort of patients with osteoarthritis and several measurements of disease severity over time will be needed to deal better with this issue.

Our study has some limitations. Firstly, our sample size was small, which may limit statistical power to detect any existing association. Secondly, radiographs were not taken for the controls. Felson *et al*¹⁵ noted that the prevalence of symptomatic knee osteoarthritis is only 9.5% of radiologically defined knee osteoarthritis. Therefore, we cannot exclude the presence of asymptomatic osteoarthritis in the controls, thus weakening the statistical power of our study. Thirdly, we examined only two polymorphisms because of their relatively high frequency. However, several additional polymorphisms have been identified in the *PPAR* γ gene,⁸ and thus these polymorphisms possibly contribute to the susceptibility to, or severity of, osteoarthritis. Finally, the control of *PPAR* γ expression and activity in vivo are complex and not well understood, and could be subject to modulation by polymorphisms in other genes. The continued accumulation of data in databases on single-nucleotide polymorphisms will help enable such studies.

In conclusion, our data suggest that the investigated polymorphisms in the *PPAR* γ gene are not associated with susceptibility to, or severity of, osteoarthritis in the French-Canadian population and therefore cannot be regarded as a major cause of osteoarthritis. These findings will require replication in patients with osteoarthritis from different ethnic populations.

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REFERENCES

- 1 **Spector TD**, MacGregor AJ. Risk factors for osteoarthritis: genetics. *Osteoarthritis Cartilage* 2004;**12**(Suppl A):S39–44.
- 2 **Fahmi H**, Pelletier JP, Martel-Pelletier J. PPAR γ ligands as modulators of inflammatory and catabolic responses on arthritis. An overview. *J Rheumatol* 2002;**29**:3–14.
- 3 **Fahmi H**, Di Battista JA, Pelletier JP, Mineau F, Ranger P, Martel-Pelletier J. Peroxisome proliferator-activated receptor gamma activators inhibit interleukin-1 β -induced nitric oxide and matrix metalloproteinase 13 production in human chondrocytes. *Arthritis Rheum* 2001;**44**:595–607.
- 4 **Cheng S**, Afif H, Martel-Pelletier J, Pelletier JP, Li X, Farrajota K, et al. Activation of peroxisome proliferator-activated receptor gamma inhibits interleukin-1 β -induced membrane-associated prostaglandin E2 synthase-1 expression in human synovial fibroblasts by interfering with Egr-1. *J Biol Chem* 2004;**279**:22057–65.
- 5 **Fahmi H**, Pelletier JP, Di Battista JA, Cheung HS, Fernandes J, Martel-Pelletier J. Peroxisome proliferator-activated receptor gamma activators inhibit MMP-1 production in human synovial fibroblasts by reducing the activity of the activator protein 1. *Osteoarthritis Cartilage* 2002;**10**:100–8.
- 6 **Bordji K**, Grillasca JP, Gouze JN, Magdalou J, Schohn H, Keller JM, et al. Evidence for the presence of peroxisome proliferator-activated receptor (PPAR) alpha and gamma and retinoid Z receptor in cartilage. PPARgamma activation modulates the effects of interleukin-1 β on rat chondrocytes. *J Biol Chem* 2000;**275**:12243–50.
- 7 **Kobayashi T**, Notoya K, Naito T, Unno S, Nakamura A, Martel-Pelletier J, et al. Pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, reduces the progression of experimental osteoarthritis in guinea pigs. *Arthritis Rheum* 2005;**52**:479–87.
- 8 **Knouff C**, Auwerx J. Peroxisome proliferator-activated receptor-gamma calls for activation in moderation: lessons from genetics and pharmacology. *Endocr Rev* 2004;**25**:899–918.
- 9 **Altman R**, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986;**29**:1039–49.
- 10 **Kellgren JH**, Lawrence JS. Radiological assessment of osteo-arthritis. *Ann Rheum Dis* 1957;**16**:494–502.
- 11 **Bellamy N**, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol* 1988;**15**:1833–40.
- 12 **Ogawa S**, Urano T, Hosoi T, Miyao M, Hoshino S, Fujita M, et al. Association of bone mineral density with a polymorphism of the peroxisome proliferator-activated receptor gamma gene: PPARgamma expression in osteoblasts. *Biochem Biophys Res Commun* 1999;**260**:122–6.
- 13 **Oh EY**, Min KM, Chung JH, Min YK, Lee MS, Kim KW, et al. Significance of Pro12Ala mutation in peroxisome proliferator-activated receptor-gamma2 in Korean diabetic and obese subjects. *J Clin Endocrinol Metab* 2000;**85**:1801–4.
- 14 **Yoo J**, Seo B, Kim Y. SNPAnalyzer: a web-based integrated workbench for single-nucleotide polymorphism analysis. *Nucleic Acids Res* 2005;**33**:W483–488.
- 15 **Felson DT**, Naimark A, Anderson J, Kazis L, Castelli W, Meenan RF. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis Rheum* 1987;**30**:914–8.