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Chapter 8

Distribution of *Peroxisome Proliferator–Activated Receptor–*γ Polymorphisms in Chinese and Dutch Patients with Inflammatory Bowel Disease

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Background: As peroxisome proliferator–activated receptor– gamma (PPAR-γ) is frequently expressed in colon, its genetic polymorphism may play a role in the etiology of inflammatory bowel disease (IBD). The aims of the present study were to determine the distribution of PPAR-γ polymorphisms Pro12Ala and C161T and to explore the association between the PPAR-γ genotypes and phenotypes of IBD patients.

Methods: A total of 244 IBD patients [212 ulcerative colitis (UC) and 32 Crohn's disease (CD)] and 220 controls in the Chinese population and 603 IBD patients (302 UC and 301 CD) and 180 controls in the white Dutch population were enrolled in the study. The phenotypes of Chinese IBD patients were grouped according to disease location. The PPAR-γ polymorphisms Pro12Ala and C161T were genotyped by PCR-based methods.

Results: In the Chinese population, T carriers of the PPAR-γ C161T polymorphism were more common in UC patients than in the controls [37.7% vs. 25.5%, odds ratio 1.77, 95% confidence interval 1.18–2.68, P = 0.007], whereas Ala carriers of the Pro12Ala polymorphism showed no significant association in UC patients, but there was a significant association of Ala carriers with more extensive disease among the UC patients (P = 0.002); Pro12Ala and C161T genotypes did not show any associations with CD patients. No associations were found for the PPAR-γ C161T SNP studied in the Dutch IBD population.

Conclusions: Our study showed the potential association between the PPAR-γ C161T polymorphism and UC patients in the central Chinese population. This finding was not replicated in the Dutch population. Further studies are necessary to explore the functional implication of the PPAR-γ C161T polymorphism in Chinese UC patients.

Inflammatory bowel disease (IBD) is a chronic, relapsing intestinal inflammatory disorder of unknown origin and is clinically classified into Crohn's disease (CD), ulcerative colitis (UC), and indeterminate colitis. Current advances suggest that an inappropriate response of a defective mucosal immune system to indigenous intestinal flora and other luminal antigens in a genetically susceptible host is at the core of this disease.[1,2] Because of its role in the regulation of colon inflammation,[3,4] peroxisome proliferator–activated receptor–gamma (PPAR-γ) can be a susceptibility gene for IBD as the NOD2 (nucleotide oligomerization domain 2)/CARD15 (caspase recruitment domain 15) gene is for CD. PPAR-γ is a member of the nuclear receptor superfamily of transcription factors. Three types of PPAR—a, c and the ubiquitously expressed b/d—have been described in mammals.[5–7] The PPAR-γ gene, on chromosome 3, band 3p25,[8] gives rise to 3 distinct mRNAs: PPAR-γ1, PPAR-γ2, and PPAR-γ3.[5,9-12]

Following activation of PPAR-γ by specific ligands, the receptor binds to its obligate heterodimer partner retinoid X receptor (RXR). Ligand binding induces a conformational change, resulting in the release of corepressors, binding of a distinct set of nuclear coactivators, and activation of gene transcription of a distinct set of nuclear coactivators, and activation of gene transcription factors.[13] Until recently, PPAR-γ was known as a receptor mainly expressed by adipose tissue and involved in the regulation of insulin resistance. PPAR-γ is activated by antidiabetic thiazolidinedione drugs.[14] In 1998, the first studies were published reporting a potential link between this receptor and intestinal diseases, originally described in colon cancer.[15–17] Apart from adipose tissue, the colon is another major tissue expressing PPAR-γ in epithelial cells.[3]

A common structural single-nucleotide polymorphism (SNP) in the PPAR-γ2 gene (Pro12Ala), a CCA-to-GCA missense mutation in codon 12 of exon B of the PPARc gene has been identified.[18] This substitution possibly results in a conformational change in protein structure and reduced function of the PPAR-γ gene. At the cellular level, reduced binding of the Ala variant to the PPAR-γ-responsive DNA elements[19] and reduced transcription of specific genes in cells overexpressing the Ala variant have been reported.[20]

The Ala allele of the common Pro12Ala polymorphism is associated with a reduced risk of type 2 diabetes.[21] This SNP also appears to have a protective effect against diabetic nephropathy.[22] Individuals with the Ala allele have also been found to have a reduced risk of colorectal cancer.[23–25] Another SNP in the PPAR-γ gene (C161T) is a silent C-to-T substitution at nucleotide [161] in exon 6 that does not cause an amino acid change.[26] The synonymous C161T polymorphism has been correlated with colorectal cancer,[27] colorectal adenoma[28] and other conditions such as psoriatic arthritis,[29] diabetic nephropathy,[30] plasma leptin levels in obese subjects,[26] extent of coronary artery disease by angiography,[31] carotid intima media

thickness,[32] and incidence of myocardial infarction among individuals younger than age 50.[33] In mice with targeted disruption of the PPAR-γ, activation of PPAR-γ expressed by both colonic epithelial cells and lamina propria macrophages has major protective effects against inflammation.[12,34] Previous studies revealed that the PPAR-γ agonist rosiglitazone was more effective in the treatment of active UC.[35–37] The discovery that 5-aminosalicylic acid (5-ASA) is a topical ligand for the PPAR-γ receptor expressed by colonic epithelial cells[38] paved the way for the rational development of novel and more powerful molecules specifically targeting intestinal PPAR-γ.

Despite the promising role of the PPAR-γ agonist, so far very few studies have assessed the role of PPARc in UC and CD patients,[35–40] and few studies have explored the possibility of the potential association of the PPAR-γ genetic polymorphism with IBD. Because PPAR-γ is frequently expressed in colonic epithelium, the common polymorphisms in the PPAR-γ gene may play a role in the etiology of IBD. Therefore, we determined the distribution of the PPAR-γ polymorphisms Pro12Ala and C161T in Chinese IBD patients and explored the potential associations between these polymorphisms and phenotypes of IBD patients in central China. We also determined the distribution of the PPAR-γ C161T polymorphism in Dutch IBD patients.

Methods

Subjects

Two independent case-control studies were performed in Chinese and Dutch populations. A total of 244 unrelated Chinese IBD patients of Han ethnicity were collected at Zhongnan Hospital of Wuhan University, Wuhan, in central China, and 603 unrelated white Dutch IBD patients were recruited from the VU University Medical Center (VUMC), Amsterdam, the Netherlands. Chinese IBD patients were classified according to age and sex [mean age 39.2 13.8 years, 140 men (57.4%) and 104 women (42.6%)] and categorized into UC [mean age 39.3 13.7 years, 122 men (57.5%) and 90 women (42.5%)] and CD [mean age 38.8 14.8 years, 18 men (56.3%)] and 14 women (43.7%)]. Dutch IBD patients were classified according to age and sex [mean age 33.1 15 years, 270 men (44.8%) and 333 women (55.2%)] and categorized into UC [mean age 32.8 14.4 years, 134 men (44.4 %) and 168 women (55.6 %)] and CD [mean age 33.3 14.5 years, 136 men (45.2%) and 165 women (54.8%)]. Diagnosis of IBD was made by conventional clinical, radiological, endoscopic, and histological criteria.[41] The patients with CD were classified by Montreal classification according to disease location (L1, terminal ileum; L2, colon; L3, ileocolon; L4, isolated upper gastrointestinal tract; and L4, a modifier that could be added to L1–L3 when concomitant upper gastrointestinal disease was present).[42,43] The patients with UC were also classified by Montreal classification according

to disease location [E1, ulcerative proctitis (proximal extent of inflammation distal to the rectosigmoid junction); E2, left-sided UC (distal UC—involvement limited to the portion of the colorectum distal to the splenic flexure); and E3, extensive UC (pancolitis—involvement extended proximal to the splenic flexure)].[42,43] All patients were longitudinally followed in the participating centers. In Chinese, 220 age and sex-matched healthy controls [mean age 37.1 11.2 years, 123 men (55.9%) and 97 women (44.1%)] were selected from the medical staff at Zhongnan Hospital of Wuhan University as well as from healthy volunteers in Wuhan city. White Dutch age-matched healthy controls [mean age 41.8 10.5 years, 84 men (46.7 %) and 96 women (53.3 %)] were recruited from healthy staff and students at the VUMC. In both study groups, the healthy controls were ethnically matched and asymptomatic with no history of malignancy and no personal or family history of IBD. Informed consent was taken from all study subjects. The study protocol was approved by the ethics committees of the participating centers.

PPAR-γ Genotyping

A blood sample was obtained from each case and healthy control and was used for DNA extraction. Genomic DNA was isolated from 5 mL of EDTA anticoagulated venous blood by conventional proteinase K digestion and the phenol/chloroform extraction method. The A260/280 ratio, as determined with a Fluorescence Spectrophotometer F-4500 (Hitachi, Tokyo, Japan), fell between 1.8 and 2.0 for all samples.

The Pro12Ala and C161T polymorphisms in the PPAR-γ gene were determined by polymerase chain reaction—restriction fragment length polymorphism (PCRRFLP) in Chinese.

For genotyping of the PPAR-γ C161T variant (dbSNP ID: rs3856806) in Chinese population, PCR was performed to detect the C161T SNP at exon 6 of the PPARc gene by using forward primer 50 -CAA GAC AAC CTG CTA CAA GC-30 and reverse primer 50 -TCC TTG TAG ATC TCC TGC AG-30 .26 PCR was done by denaturing the samples of genomic DNA at 94 C for 1 minute, followed by 34 cycles, each consisting of denaturing at 94 C for 30 seconds, annealing at 56 C for 30 seconds, and an extension at 72 C for 1 minute. The thermal cycles finished with a final extension at 72 C for 5 minutes. The protocol for digestion of PCR products was carried out in a 20-μL volume. A master mix was made in multiples of 3 units of Eco72I, an isoschizomer of PmlI (Fermentas Int. Inc., Burlington, Ontario, Canada), 10 μL of PCR product, 2 μL of 10 Buffer Tango, and 7 μL nuclease-free water for each reaction, and then this master mix was added to all samples. The PCR product was digested overnight with Eco72I at 37 C. The digested PCR products were run in 2.5% agarose gel for 30 minutes and stained with ethidium bromide. This resulted in 2 fragments (120 and 80 bp) for the wild type, and 1 fragment (200 bp) when the restriction site was eliminated by the C161T transition. The genotypes were identified as C/C, C/T, and T/T.

For genotyping of the PPAR-γ Pro12Ala variant (dbSNP ID: rs1801282) in the Chinese population, a 270bp fragment of the PPAR-γ gene encompassing the site of the polymorphism was generated from genomic DNA by PCR using forward primer 50 -GCC AAT TCA AGC CCA GTC-30 and reverse primer 50 -GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G-30 .[18]

PCR was performed as follows: initial denaturing at 94 C for 5 minutes; followed by 35 amplification cycles, each consisting of denaturing at 94 C for 30 seconds, annealing at 51 C for 40 seconds, and an extension at 72 C for 40 seconds; and a final extension at 72 C for 4 minutes. The protocol for digestion of PCR products was carried out in a 20-μL volume. A master mix was made in multiples of 10 units of Bsh1236I, an isoschizomer of BstUI (Fermentas Int. Inc., Burlington, Ontario, Canada), 10 μL of PCR product, 2 μL of 10 Buffer R, and 7 μL of nuclease-free water for each reaction, and then this master mix was added to all samples. The PCR product was digested overnight with Bsh1236I at 37 C. The DNA fragments were then separated on 2.5% agarose gel electrophoresis and stained with ethidium bromide. After digestion, the products with Bsh1236I were a 270-bp fragment representing Pro/Pro (C/C) genotype, 2 fragments of 227 and 43 bp indicating the Ala/Ala (G/G) genotype, and 3 fragments of 270, 227, and 43 bp, indicating the Pro/Ala (C/G) genotype.

For quality control purposes, negative and positive controls were processed with each batch of samples and all experiments were repeated twice to ensure consistency. Positive controls consisted of 20% of samples with each of the genotypes, as determined by sequencing of purified PCR products using an ABI 3730 apparatus (Applied Biosystems, Foster City, CA). Genotyping of the PPAR-γ C161T SNP in Dutch population was performed with appropriate quality control measures by the Taqman method at the VU University Medical Center in Amsterdam with an ABI PRISM 7000

Sequence detection System (Applied Biosystems, Foster City, CA) with primers and probes designed using Primer Express software (version 2.0). Primers used were 5'-CCA GAAAATGACAGACCTCAGACA-3' and 5'-GCAGGA GCGGGTGAAGACT-3' and MGB probes 6FAM-TCACG GAACACGTGCA and VIC-ACGGAACATGTGCAGC.

Statistical Analysis

The statistical significance of the differences between cases and controls was tested by the chi-square (v2) or Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were computed according to Woolf's formula. All analyses were adjusted for age and sex. The contribution of polymorphisms to the disease location of UC and CD patients was also estimated by contingency-table chi-square analysis. A P value < 0.05 was considered statistically significant. Each polymorphism was tested to ensure that it fit Hardy-Weinberg equilibrium. Power calculations were done with Quanto software for the SNP PPAR-γ C161T.[44]

Results

Susceptibility Studies in Han Chinese and Dutch Whites

The distribution of the PPAR-γ Pro12Ala and C161T genotypes and alleles in Han Chinese UC, CD, and controls is shown in Table 1. In all subjects, genotype distributions were in Hardy-Weinberg equilibrium for either the Pro12Ala (P = 0.680 in UC, 0.781 in CD, and 0.079 in controls) or C161T (P = 0.131 in UC, 0.591 in CD, and 0.107 in controls) polymorphism. For the Pro12Ala polymorphism, the Pro/Pro, Pro/Ala, and Ala/Ala genotype frequencies were 89.2%, 10.3%, and 0.5%, respectively, in the UC patients compared with 90.0%, 9.1% and 0.9%, respectively, for the controls. No statistically significant differences were observed in the PPAR-γ Pro12Ala genotype and allele frequencies between UC patients and controls (Table 1). For the C161T polymorphism, the C/C, C/ T, and T/T genotype frequencies were 62.3%, 35.4%, and 2.3%, respectively, in the UC patients compared with 74.5%, 25.0%, and 0.5%, respectively, for the controls. The prevalence of the C/T genotype tended to be higher in UC patients than in controls, reaching a significant difference (35.4% vs. 25.0%, OR = 1.69, 95% CI = 1.12–2.57, P = 0.015). When combining the C/T and T/T genotypes, the frequency of T carriers was higher in UC patients than in controls (37.7% vs. 25.5%, OR = 1.77, 95% CI = 1.18–2.68, P = 0.007). Similarly, the frequency of the T allele was increased in UC patients when compared with the controls (20.1% vs. 12.9%, OR = 1.68, 95% CI = 1.17–2.43, P = 0.006). No other significant differences were observed in the PPAR-γ Pro12Ala and C161T genotypes and allele frequencies between CD patients and controls (Table 1).

To replicate the identified association in the Han Chinese population in another independent cohort, we genotyped the C161T SNP in the Dutch white population.

Table 2 shows the distribution of genotypes and alleles of the C161T SNP in white Dutch UC and CD patients and controls. There were no statistically significant deviations from Hardy-Weinberg equilibrium for the C161T SNP (P = 0.832 in controls, 0.381 in UC, and 0.511 in CD). For the C161T polymorphism, the C/C, C/T, and T/T genotype frequencies were 76.2%, 22.8%, and 1.0%, respectively, in the UC patients, 77.4%, 21.6%, and 1.0%, respectively, in the CD patients, and 74.4%, 23.9%, and 1.7%, respectively, in the controls. There were no significant differences in the genotype or allelic distribution or the frequency of T carriers (combining C/T and T/T genotypes) between the UC and CD patients and controls.

Table 1. Distribution of PPAR-γ Pro12Ala and C161T Genotypes and Allele Frequencies in Chinese UC, CD, and Healthy Controls

	Healthy Controls	UC			CD		
	n = 220 (%)	n = 212 (%)	OR* (95% CI†)	P	n = 32 (%)	OR (95% CI)	P
Pro12Ala							
Pro/Pro	198 (90.0%)	189 (89.2%)	1.00 (reference)		29 (90.6%)	1.00 (reference)	
Pro/Ala	20 (9.1%)	22 (10.3%)			3 (9.4%)		
Ala/Ala	2 (0.9%)	1 (0.5%)			0		
Ala carrier‡	22 (10.0%)	23 (10.8%)	1.09 (0.59–2.03)	0.875	3 (9.4)	0.93 (0.26–3.31)	0.961
Pro	416 (94.5%)	400 (94.3%)	1.00 (reference)		61 (95.3%)	1.00 (reference)	
Ala	24 (5.5%)	24 (5.7%)	1.04 (0.58–1.86)	0.896	3 (4.7%)	0.85 (0.25–2.92)	0.853
C161T							
C/C	164 (74.5%)	132 (62.3%)	1.00 (reference)		24 (75.0%)	1.00 (reference)	
C/T	55 (25.0%)	75 (35.4%)			7 (21.9%)		
T/T	1 (0.5%)	5 (2.3%)	1 (3.1%)				
T carrier§	56 (25.5%)	80 (37.7%)	1.77 (1.18–2.68)	0.007	8 (25.0%)	0.98 (0.41–2.30)	0.977
C	383 (87.1%)	339 (79.9%)	1.00 (reference)		55 (85.9%)	1.00 (reference)	
T	57 (12.9%)	85 (20.1%)	1.68 (1.17–2.43)	0.006	9 (14.1%)	1.10 (0.52–2.35)	0.843

Odds ratio; †confidence interval; ‡Ala carrier = Pro/Ala or Ala/Ala; *T carrier = C/T or T/T.

Table 2. Distribution of PPAR-γ C161T Genotypes and Allele Frequencies in White Dutch UC, CD, and Healthy Controls

		UC			CD		
	Healthy controls, n = 180 (%)	n = 302 (%)	OR* (95% CI†)	P	n = 301 (%)	OR (95% CI)	P
C161T							
C/C	134 (74.4%)	230 (76.2%)	1.00 (reference)		233 (77.4%)	1.00 (reference)	
C/T	43 (23.9%)	69 (22.8%)			65 (21.6%)		
T/T	3 (1.7%)	3 (1.0%)			3 (1.0%)		
T carrier‡	46 (25.6%)	72 (23.8%)	0.9 (0.59–1.40)	0.754	68 (22.6%)	0.9 (0.56–1.30)	0.529
C	311(86.4%)	529 (87.6%)	1.00 (reference)		531 (88.2%)	1.00 (reference)	
T	49 (13.6%)	75 (12.4%)	0.9 (0.61–1.32)	0.663	71 (11.8%)	0.85 (0.57–1.25)	0.469

*Odds ratio; †confidence interval; ‡T carrier = C/T or T/T.

Genotype-Phenotype Studies in Han Chinese

The associations of the PPAR-γ Pro12Ala genotype with disease location in UC and CD patients are shown in Table 3. There was a significant association between the PPAR-γ Pro12Ala genotype and disease location in UC patients. The Ala carriers were more frequent among UC patients with extensive colitis than among those with less extensive forms (P = 0.002). However, the relation of Ala carriers with disease location among CD patients was

not significant. The associations of the PPAR-γ C161T genotype with disease location in UC and CD patients are shown in Table 4. The PPAR-γ C161T genotype was not significantly associated with disease location in UC and CD patients (Table 4).

Table 3. Association of PPAR-γ Pro12Ala genotype with Disease Location in Chinese UC and CD Patients

	Pro12Ala				
	Pro/Pro	Pro/Ala	Ala/Ala	Ala carrier	P*
UC: Location of disease					0.002
E1: ulcerative proctitis, n† = 73 (%)	71 (97.3%)	2 (2.7%)	0 (0.0%)	2 (2.7%)	
E2: left sided UC (distal UC), n = 76 (%)	68 (89.5%)	8 (10.5%)	0 (0.0%)	8 (10.5%)	
E3: extensive UC (pancolitis), n = 63 (%)	50 (79.4%)	12 (19.0%)	1 (1.6%)	13 (20.6%)	
CD: Location of disease					0.512
L1: terminal ileum, n = 8 (%)	8 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
L2: colon, n = 10 (%)	8 (80.0%)	2 (20.0%)	0 (0.0%)	2 (20.0%)	
L3: ileocolon, n = 13 (%)	12 (92.3%)	1 (7.7%)	0 (0.0%)	1 (7.7%)	
L4: Isolated upper gastrointestinal tract, n = 1 (%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

*For comparison between Pro/Pro genotype and Ala carrier (Pro/Ala or Ala/Ala genotypes); †number of patients in each subgroup with percentage between brackets.).

Table 4. Association of PPAR-γ C161T Genotype with Disease Location in Chinese UC and CD Patients

	C161T				
	C/C	C/T	T/T	T carrier	P*
UC: Location of disease					0.786
E1: ulcerative proctitis, n† = 73 (%)	47 (64.4%)	24 (32.9%)	2 (2.7%)	26 (35.6%)	
E2: left sided UC (distal UC), n = 76 (%)	45 (59.2%)	30 (39.5%)	1 (1.3%)	31 (40.8%)	
E3: extensive UC (pancolitis), n = 63 (%)	40 (63.5%)	21 (33.3%)	2 (3.2%)	23 (36.5%)	0.174
CD: Location of disease					
L1: terminal ileum, n = 8 (%)	7 (87.5%)	0 (0.0%)	1 (12.5%)	1 (12.5%)	
L2: colon, n = 10 (%)	5 (50.0%)	5 (50.0%)	0 (0.0%)	5 (50.0%)	
L3: ileocolon, n = 13 (%)	11 (84.6%)	2 (15.4%)	0 (0.0%)	2 (15.4%)	
L4: isolated upper gastrointestinal tract, n = 1 (%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

*For comparison between C/C genotype and T carrier (C/T or T/T genotypes); †number of patients in each subgroup with percentage in parentheses.

Discussion

Although there has been an emerging interest in the role of PPAR-γ as the new target in the regulation of gut inflammation, relatively limited information exists to date on the relationship between IBD and PPAR-γ genetic polymorphisms. In this study, we investigated the distribution of the PPAR-γ polymorphisms Pro12Ala and C161T in Chinese IBD patients and the C161T polymorphism in white Dutch IBD patients and ethnically matched healthy controls. T carriers (C/T or T/T genotype) of the C161T polymorphism were found to be associated with a significant 1.77-fold (95% CI = 1.18–2.68) increase in the OR in the UC patients compared with the C/C genotype in the Chinese. No other studies in the Chinese on the C161T polymorphism in relation to UC have been published thus far to compare our data; however, a study done in an Indian population of colorectal cancer patients showed similar results (OR = 1.61, 95% CI = 1.10–2.36).[27] The comparable result in both populations might suggest a potential link between the patients of UC and colorectal cancer by means of the C161T PPAR-γ polymorphism. Our study could not find a significant association of the Ala allele (G allele) with UC patients, which again conforms with the results of a study of colorectal cancer patients in the Indian population but contrasts with case-control studies in Spanish[23] and Singaporean[25] populations, which showed the Ala allele to have a potentially protective role in colorectal cancer patients.

PPAR-γ SNPs have been recently genotyped in the Dutch IBD population.[45] However, the synonymous PPARc C161T SNP was not among the 8 SNPs successfully genotyped. Moreover, HapMap data show that the strongest linkage disequilibrium (LD) of this SNP was moderate with SNP rs2120825 (r2 = 0.41 and D0 = 0.63; EUR in HapMap rel24/phaseII Nov08, on NCBI B36 assembly, dbSNP b126), and a proxy of the PPAR-γ C161T SNP was not genotyped in that study.

In the current study of Dutch white patients with UC, we were not able to replicate the association between carriage of the minor allele of the C161T polymorphism and UC observed in the Chinese patients. This cannot be explained by differences in disease location because the distribution of genotypes is similar between the 3 Dutch UC patient subgroups (data not shown) and also does not differ between the 3 Chinese UC patients subgroups (Table 4). To evaluate whether insufficient power caused the lack of replication, we performed a power calculation with the following assumptions and parameters: minor-allele frequency for allele T of rs3856806 of 0.136, sample size of 302 cases and case-control ratio of 0.6, dominant inheritance model, and disease prevalence of UC (0.3/1000). The odds ratio was fixed at 1.77 (OR in Chinese UC). Assuming these parameters, our study had 79.6% power to detect an association with an OR 1.77 or greater at a 5% significance level. However, if we consider the lower boundary of the 95% confidence interval for the odds ratio, the RR to be reached

would be 1.18. Moreover, the phenomenon of ''winner's curse,'' effect sizes reported initially are often overestimates of the true effect size found in subsequent studies, should be taken into account. Our replication study would then be underpowered and a much larger number of cases and controls required for replication.

The ethnic diversity between the 2 samples of Chinese and Dutch origins could also be responsible for the observed difference between UC patients. Alternatively, the causative variant allele was in strong LD with the PPAR-γ 161T allele in the Chinese but not the Dutch population. In addition, conflicting results might be a result of different clinical pathologies, possibly resulting from the interaction of genetic (modifier) genes with varying environmental factors, for example, commensal intestinal flora.

We also did not find this SNP to be associated with CD in the Dutch population. This is in line with our previous larger study, which did not demonstrate a significant association of any of 8 tagging SNPs with UC and CD.[45]

The association study of PPAR-γ polymorphisms with CD patients did not show any significant relation in either the Chinese or Dutch population, the statistical power of which was weakened by the small sample size of Chinese in our study.

The PPAR-γ Pro12Ala has been the most investigated PPAR-γ polymorphism, but there have been very few studies of PPAR-γ C161T polymorphism in relation to gastrointestinal diseases. It was shown in previous studies that the impaired expression of PPAR-γ in UC was affected by commensal intestinal flora.39 Our study showed a potential influence of the PPAR-γ C161T polymorphism on Chinese UC patients, and it is plausible that the C161T polymorphism may contribute to or account for the impaired PPAR-γ expression in Chinese UC patients in addition to the bacteria. We also found that in the Chinese population the PPAR-γ Pro12Ala polymorphism did not seem to increase the risk of UC or CD but that Ala carriers in UC patients may be associated with a more extensive form of the disease.

In conclusion, we report for the first time the potential association between the C161T polymorphism in the PPAR-γ and UC patients in the Chinese population. However, further studies with a larger sample size in independent cohorts from the same ethnic group are necessary to determine the relation of the PPAR-γ C161T polymorphism with IBD patients.

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