ORIGINAL PAPERS

Common *PPARγ* variants C161T and Pro12Ala are not Associated with Inflammatory Bowel Disease in an Australian Cohort

Georgia E Hume[1,2], Elizabeth V Fowler[1], Lyn R Griffiths[3], James D Doecke[4], Graham L Radford-Smith[1,2,5]

1) Inflammatory Bowel Diseases Laboratory, Queensland Institute of Medical Research, Herston; 2) Department of Gastroenterology and Hepatology, Royal Brisbane & Womens Hospital, Herston; 3) Genomics Research Centre, Griffith Institute for Health and Medical Research, Griffith University, Gold Coast, Queensland; 4) CSIRO Preventative Health Flagship, CSIRO Mathematics, Informatics and Statistics, RBWH Herston, Queensland; 5) School of Medicine, University of Queensland, Herston, Queensland, Australia

Abstract

Background & Aims: Peroxisome proliferator-activated receptor (PPAR) γ is a transcription factor, highly expressed in colonic epithelial cells, adipose tissue and macrophages, with an important role in the regulation of inflammatory pathways. The common *PPARγ* variants C161T and Pro12Ala have recently been associated with Ulcerative Colitis (UC) and an extensive UC phenotype respectively, in a Chinese population. *PPARγ* Pro12Ala variant homozygotes appear to be protected from the development of Crohn's disease (CD) in European Caucasians. **Methods**: A case-control study was performed for both variants (CD n=575, UC n=306, Controls n=360) using a polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis in an Australian IBD cohort. A transmission disequilibrium test was also performed using CD trios for the *PPARγ* C161T variant. Genotype-phenotype analyses were also undertaken. **Results**: There was no significant difference in genotype distribution data or allele frequency between CD and UC patients and controls. There was no difference in allele transmission for the C161T variant. No significant relationship between the variants and disease location was observed. **Conclusions**: We were unable to replicate in a Caucasian cohort the recent association between *PPARγ* C161T and UC or between *PPARγ* Pro12Ala and an extensive UC phenotype in a Chinese population. There are significant ethnic differences in genetic susceptibility to IBD and its phenotypic expression.

Received: 20.08.2012 Accepted: 12.09.2012
J Gastrointestin Liver Dis
December 2012 Vol. 21 No 4, 349-355
Address for correspondence: Georgia E Hume
IBD Laboratory, QIMR
Herston Rd, Herston
Queensland, 4029
Australia
E-mail: georgiaH@qimr.edu.au

Key words

Genetic polymorphism – *PPARγ* – inflammatory bowel disease – Crohn's disease – ulcerative colitis

Introduction

Peroxisome proliferator-activated receptor (PPAR) γ is highly expressed in colonic epithelial cells and is an effective therapeutic target for *PPARγ* agonists including 5-aminosalicylate (5-ASA) in human ulcerative colitis (UC) [1]. PPARs are transcription factors, members of the nuclear hormone receptor subfamily which regulate transcription of other genes by binding to specific DNA sequence elements, peroxisome proliferator response elements (PPREs). PPARγ is one of three known subtypes with a pivotal role in adipocyte differentiation and glucose homeostasis. However, PPARγ also controls the expression of genes involved in inflammation and cell proliferation. It is bound and activated by naturally occurring polyunsaturated fatty acids and prostaglandins, as well as by synthetic compounds such as thiazolidinediones and non-steroidal anti-inflammatory drugs [2]. The highest expression of PPARγ is observed in adipose tissue, macrophages and colonic epithelium, all of which express all three PPARγ subtypes [3].

The inflammatory bowel diseases are characterized by high levels of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and activation of the nuclear factor- kappa β (NF-κB) and c-Jun NH2-terminal kinase (JNK)/p38 mitogen-activated protein kinase (MAPK) pathways. Both these important signaling pathways are down regulated by PPARγ activation [4]. In the dextran sodium sulfate (DSS) mouse model of colitis, treatment with PPARγ ligands, including the thiazolidinedione troglitazone, resulted in a significant reduction in inflammation and disease severity [5]. Selective PPARγ and retinoid x receptor (RXR) agonists have also been shown to significantly reduce trinitrobenzene sulfonic acid (TNBS)-induced

colitis in a mouse model [6]. Administration of the short chain fatty acid butyrate is therapeutic in IBD and PPARγ plays an important role in butyrate-mediated inhibition of lipopolysaccharide (LPS)-induced NF-κB activation [7]. More recently in human studies, rosiglitazone, a thiazolidinedione has been efficacious in the treatment of mild-moderate UC in a multicenter, randomized, double blind, placebo-controlled US study of 105 patients [8]. Rosiglitazone also demonstrated therapeutic efficacy in mild-moderate UC when combined with 5-aminosalicylate in a trial of 42 Chinese patients [9].

PPARγ in the colon is predominantly expressed in the epithelial surface layer. Importantly, UC patients have very low levels of PPARγ expression in colonic epithelium in both inflamed and non-inflamed tissue. Levels are however normal in the colonic mucosa in Crohn's disease (CD), in both the inflamed and non-inflamed state. The mechanism for this difference is unknown [10]. Toll-like Receptor 4 (TLR4), the LPS receptor, is able to regulate PPARγ expression, possibly as a negative feedback loop to regulate the inflammatory process when stimulated by gram negative bacteria in the gut. Therefore in UC where TLR4 is upregulated and the expression of PPARγ is impaired, there may be loss of tolerance to colonic bacteria and subsequent chronic inflammation [4, 10].

It has also been demonstrated that PPARγ and TNFα are over expressed by mesenteric adipocytes in patients with CD [11]. In view of its role in lipogenesis, dysregulated PPARγ expression could contribute to the hypertrophy of mesenteric white adipose tissue. This in turn increases TNFα production contributing to the local inflammatory response and the characteristic mucosal ulcerations of the mesenteric border in CD. In addition, PPARγ is markedly upregulated in activated macrophages where it antagonizes several pro-inflammatory pathways. Hence PPARγ agonists may have a therapeutic role in inflammatory disorders including atherosclerosis, rheumatoid arthritis and CD [12]. PPARγ is also involved in immunoregulation by controlling helper T cell responses as well as having an important role in regulatory T cell (Treg) function [13, 14]. *PPARγ* has previously been identified as a susceptibility gene in the SAMP1/YitFc Mouse model of CD while rare *PPARγ* alleles have been associated with CD in humans [15].

In view of these findings, there has been recent interest in the potential role of common mutations in the *PPARγ* gene in the inflammatory bowel diseases. A C→G variant in exon 1 of the *PPARγ* gene (rs1801282), results in the substitution of Alanine for Proline at position 12 (Pro12Ala) in *PPARγ2* [16]. The variant shows decreased binding affinity to the cognate promoter element and a reduced ability to transactivate responsive promoters. It has been associated with decreased receptor activity, lower BMI and improved insulin sensitivity [16]. Recently, the variant allele has been associated with an extensive UC phenotype in a Chinese IBD population [17]. However, a recent meta-analysis suggested that European Caucasian patients are afforded protection from CD by the AlaAla genotype [18]. A small Turkish study of the *PPARγ* Pro12Ala variant could not demonstrate any association with the development of IBD [19]. A second frequent polymorphism at position 161 in exon 7 of the *PPARγ* gene (rs 3856806) results from a silent C→T substitution. It has been associated with altered plasma leptin levels in obese humans, as well as a reduced coronary artery disease risk [20, 21]. The variant T allele has been associated with UC in a Chinese population although this finding was not replicated in a Dutch UC population. No specific phenotypic associations were observed [17]. The variant T allele also appeared to afford protection from the development of colonic adenomas in a Dutch population [22]. At odds with this result is the observation of an association between the variant allele and colorectal cancer in an Indian population [23]. The gene map locus is 3p25, some distance from the IBD 9 locus at 3p26 [24]. More recently, a genome wide association study has identified 3p21 as a locus of interest in CD [25].

In view of the physiological characteristics of PPARγ, the therapeutic efficacy of PPARγ agonists in IBD, as well as the recent association of *PPARγ* C161T with UC in a Chinese population and *PPARγ* Pro12Ala with protection from CD in European Caucasians, we report our own investigation into the role of the common *PPARγ* mutations C161T and Pro12Ala in a well-characterised Australian IBD cohort.

Patients and methods

Subjects

This candidate gene association study of case–control design involved Australian Caucasian patients recruited from the IBD Clinical and Research Programme at the Royal Brisbane & Women's Hospital (RBWH), Brisbane, Queensland, Australia (CD, n = 575; UC, n = 306) as previously described [26]. Informed consent was obtained from all patients and the RBWH Human Research Ethics Committee approved the study protocol. Patients with CD were a consecutive series of patients prospectively recruited into a longitudinal IBD research protocol from 1994. The RBWH is the major IBD referral centre for north Brisbane, which encompasses a population of approximately 800,000. All cases are recorded on an IBD database, together with relevant phenotypic information including disease distribution, behaviour and duration, surgery, smoking and histological data such as granulomas. Phenotypic characteristics were carefully determined by two investigators using clinical, endoscopic, radiological, histological and surgical data available in the patient's clinical records. The investigators were blinded to the patient's genotype during this process. CD phenotyping was based on the Montreal Classification but with disease behaviour reclassified as stricturing or non-stricturing independent of the presence of penetrating disease [27]. Controls (n =360) were healthy Caucasian subjects recruited from the east coast of Australia over a 4-year period through the Genomics Research Centre (Griffith University, Queensland, Australia) as previously described [26].

Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells using the salting-out technique [28]. Both polymorphisms were detected using a polymerase chain reaction (PCR)-restriction fragment polymorphism analysis. For the *PPARγ* P12A polymorphism, the PCR was carried out using Platinum PCR Supermix (Invitrogen Australia, Mount Waverley, Victoria, Australia) and the primers 5' GCC AAT TCA AGC CCA GTC 3' and 5' GAT ATG TTG CAG ACA GTG TAT CAG TGA AGG AAT CGC TTT CCG 3'. After amplification (annealing temperature 62^0C) the product was digested using BstU1 (New England Biolabs, Genesearch, Arundel, Queensland, Australia). The enzyme cuts the product when the variant allele is present, allowing the two alleles to be identified on a 2.8% agarose gel. For the *PPARγ* C161T mutation, genotyping included PCR (annealing temperature 56^0C) with the primers 5' CAA GAC AAC CTG CTA CAA GC 3' and 5' TCC TTG TAG ATC TCC TGC AG 3', followed by digestion with HpyCH4 IV (New England Biolabs). HpyCH4IV cuts the product in the presence of the wild-type allele hence the two alleles can be easily distinguished on a 2.5% agarose gel, with ethidium bromide for visualisation.

Statistical analysis

We compared the frequency of the two *PPARγ* polymorphisms between CD patients, UC patients and controls using the Chi Square test; Fisher's Exact p-values were utilized where necessary (Table I). Further stratifications were conducted for disease location for both CD and UC patients (Tables II, III), comparing control genotype to each individual sub group. Allele associations were also compared. Two tailed p-values of <0.025 were considered statistically significant (Bonferroni adjusted for multiple comparisons). All statistical analyses were conducted using the R statistical software package (R Development Core Team, R: A Language and Environment for Statistical Computing, Vienna, Austria.). Haploview [28] was used to analyse HapMap SNP genotype data (EUR in HapMap rel24/phaseII Nov08, on NCBI B36 assembly, dbSNP b126).

Results

Genotype and allele distribution

The genotype distribution data were similar between the control group and overall IBD cases for both *PPARγ* Pro12Ala (p=0.97) and C161T (p=0.34). The allele frequency of the variant T allele of *PPARγ* C161T was higher in the CD group compared to controls but this did not meet statistical significance (0.142 vs 0.115 p=0.09). The variant T allele frequency in the UC group was similar to controls (0.128 vs 0.115 p=0.72). The allele frequencies for the *PPARγ* Pro12Ala variant G allele did not vary significantly between the control group and UC (0.128 vs 0.108 p=0.33) or CD (0.128 vs 0.133 p=0.71) (Table I). In all the study groups, *PPARγ* C161T and Pro12Ala were in Hardy-Weinberg Equilibrium.

Table I. Genotype distribution and allele frequency data for *PPARγ*C161T (rs3856806) and Pro12Ala (rs1801282)

IBD phenotype	**rs1801282** Genotype	Control N	Case N	Genotype Control	Frequency Case	Genotype p-value	Allele p-value
Overall UC association	CC	205	243	0.76	0.79		
	CG	61	60	0.23	0.20		
	GG	4	3	0.01	0.01	0.61	
	Minor allele			0.128	0.108		0.33
	rs3856806 Genotype	Control N	Case N	Genotype Control	Frequency Case	Genotype p-value	Allele p-value
	CC	284	233	0.79	0.77		
	CT	71	64	0.20	0.21		
	TT	6	7	0.02	0.02	0.56	
	Minor allele			0.115	0.128		0.72
	rs1801282 Genotype	Control N	Case N	Genotype Control	Frequency Case	Genotype p-value	Allele p-value
Overall CD association	CC	205	432	0.76	0.75		
	CG	61	133	0.23	0.23		
	GG	4	10	0.01	0.02	0.93	
	Minor allele			0.128	0.133		0.71
	rs3856806 Genotype	Control N	Case N	Genotype Control	Frequency Case	Genotype p-value	Allele p-value
	CC	284	422	0.79	0.74		
	CT	71	134	0.20	0.24		
	TT	6	14	0.02	0.02	0.25	
	Minor allele			0.115	0.142		0.09

Table II. CD Disease location genotype distribution and allele frequency data for *PPARγ*C161T (rs3856806) and Pro12Ala (rs1801282)

Phenotype	**rs1801282** Genotype	Control N	Case N	Genotype Control	Frequency Case	Genotype p-value	Allele p-value
Location							
Ileal	CC	205	174	0.76	0.79		
	CG	61	43	0.23	0.19		
	GG	4	4	0.01	0.02	0.68	
	Minor allele			0.128	0.115		0.56
Colonic	CC	205	46	0.76	0.82		
	CG	61	10	0.23	0.18		
	GG	4	0	0.01	0.00	0.46	
	Minor allele			0.128	0.089		0.26
Ileocolonic	CC	205	24	0.76	0.67		
	CG	61	11	0.23	0.31		
	GG	4	1	0.01	0.03	0.46	
	Minor allele			0.128	0.181		0.22
Location	**rs3856806**						
Ileal	CC	284	164	0.79	0.75		
	CT	71	47	0.20	0.21		
	TT	6	8	0.02	0.04	0.26	
	Minor allele			0.115	0.144		0.15
Colonic	CC	284	43	0.79	0.78		
	CT	71	11	0.20	0.20		
	TT	6	1	0.02	0.02	0.99	
	Minor allele			0.115	0.118		0.92
Ileocolonic	CC	284	121	0.79	0.76		
	CT	71	36	0.20	0.23		
	TT	6	2	0.02	0.01	0.71	
	Minor allele			0.115	0.126		0.62

Transmission disequilibrium test

Ninety-nine CD trios were genotyped for the *PPARγ* C161T mutation in view of the findings from the case control analysis. Of these, there were 44 informative allele transmissions from a non-affected parent to an index CD case. There was no difference in allele transmission between the wild-type and variant with 50% transmission of each allele.

Genotype phenotype relationships

There was no significant difference in genotype distribution or allele frequency for *PPARγ* Pro12Ala or C161T between the CD disease locations, ileum (L1), colon (L2) and ileocolon (L3) (Table II). Similarly, we were unable to demonstrate any difference in allele frequency or genotype distribution for either *PPARγ* mutation when considering disease extent in UC (left-sided vs extensive, Table III). The variant T allele for *PPARγ* C161T was more frequent in patients with an extensive UC phenotype however this did not meet statistical significance (0.142 vs 0.115; p=0.26).

PPARγ HapMap data

Analysis of *PPARγ*HapMap data in Haploview revealed that rs3856806 was not included in any haplotype block identified in this gene, although it does show moderate linkage disequilibrium (LD; 57-72%) with several SNPs in the first haplotype block (including rs1801282; LOD = 4.3, r2 = 0.29, D'=0.63) (Data not shown). Haplotype block 1 spans 73kb at the 5' end of the gene, and this region also contains four intronic *PPARγ* SNPs, which are in LD with rs3856806 (but not block 1) despite being physically distant (115-134kb apart). The rs1801282 SNP is in strong LD with several SNPs in haplotype block 1.

Discussion

This case control study did not demonstrate any significant association between the two common *PPARγ* mutations C161T and Pro12Ala and IBD in an Australian patient cohort. We were unable to replicate the recently reported association between the variant C161T allele and a Chinese UC population, despite larger patient and control numbers. This confirms the lack of replication in a Dutch UC population of similar numbers. There was also no evidence that the *PPARγ* AlaAla genotype afforded any protection from the development of CD. It is notable that the genotype frequencies for C161T were similar between Australian UC cases and controls, Dutch UC Cases and controls and

Table III. UC Disease extent genotype distribution and allele frequency data for PPARγC161T (rs3856806) and Pro12Ala (rs1801282)

Phenotype	**rs1801282** Genotype	Control N	Case N	Genotype Control	Frequency Case	Genotype p-value	Allele p-value
Location							
Left-sided disease	CC	205	66	0.76	0.73		
	CG	61	23	0.23	0.26		
	GG	4	1	0.01	0.01	0.83	
	Minor allele			0.128	0.139		0.70
Extensive disease	CC	205	98	0.76	0.78		
	CG	61	26	0.23	0.21		
	GG	4	1	0.01	0.01	0.78	
	Minor allele			0.128	0.112		0.53
Location	**rs3856806**						
Left-sided disease	CC	284	71	0.79	0.79		
	CT	71	15	0.20	0.17		
	TT	6	4	0.02	0.04	0.24	
	Minor allele			0.115	0.128		0.63
Extensive disease	CC	284	90	0.79	0.73		
	CT	71	31	0.20	0.25		
	TT	6	2	0.02	0.02	0.43	
	Minor allele			0.115	0.142		0.26

Table IV. Genotype distribution data for PPARγC161T (rs3856806) in Australian, Dutch and Chinese UC patients and Controls.

	Australian	Australian	Dutch	Dutch	Chinese	Chinese
	UC	Controls	UC	Controls	UC	Controls
PPARY	n = 304	n = 361	n = 302	n = 180	n = 212	n = 220
C161T						
C/C	233 (76.6%)	284 (78.7%)	230 (76.2%)	134 (74.4%)	132 (62.3%)	164 (74.5%)
C/T	64 (21.1%)	71 (19.7%)	69 (22.8%)	43 (23.9%)	75 (35.4%)	55 (25.0%)
T/T	7 (2.3%)	6 (1.7%)	3 (1.0%)	3 (1.7%)	5 (2.3%)	1 (0.5%)

Chinese controls. However, the Chinese UC patients had a significantly different genotype distribution (Table IV) [17]. It is possible that this represents real variation in disease pathogenesis between Caucasian and Chinese patients. It is well recognized that the major CD susceptibility mutations in *NOD2/CARD15, IL23R* and *ATG16L1* do not contribute significantly to disease susceptibility in Asian patients [13, 14]. Specifically, mutations in *NOD2/CARD15* are exceedingly rare in Chinese patients [29]. In contrast, SNPs within the tumour necrosis factor super family member 15 *(TNFSF15)* gene have been significantly associated with CD in both Japanese and European IBD populations [27]. Hence it is apparent that genetic susceptibility to IBD will vary markedly between different ethnic groups, however some susceptibility loci will be shared. In addition, the phenotype of UC varies considerably between Asian and Caucasian patients. Chinese patients predominantly present with distal or left-sided disease and have a later age of disease onset [30]. Caucasian UC patients are more likely to have extensive colitis, a positive family history and a negative correlation with cigarette smoking [8]. It is evident therefore, that both genetic and environmental factors involved in the pathogenesis of the disease will vary significantly between Chinese and Caucasian subjects.

The role of genetics in IBD susceptibility is supported by epidemiological data and appears to be stronger in CD than UC. The greatest risk factor for developing disease is having an affected relative, particularly a sibling which results in a 13-36 times increased risk of CD, and a 7-17 times increased risk of UC. In addition, concordance rates among monozygotic twins are significantly higher than among dizygotic twins and are higher in CD than in UC [31, 32]. This makes the demonstration of association between a particular gene mutation and UC even more difficult. It is only recently with the advent of genome-wide association studies (GWAS) capable of genotyping very large numbers of patients for thousands of single nucleotide polymorphisms (SNPs) that multiple clear susceptibility loci for UC have been identified [9, 33]. It will clearly be most informative to compare the results of GWAS conducted using Chinese

patients to those in Caucasian populations to more accurately determine major ethnic genetic differences. Most recently, a GWAS conducted in Japanese UC patients did not identify *PPARγ* C161T (rs3856806) as a site of significant disease association [30].

Whilst the allele frequency for the C161T variant was higher in CD patients than controls, this result did not achieve statistical significance. A lack of association was supported by a lack of transmission disequilibrium between the wild-type and variant alleles when studying family trios for this mutation. In addition, GWAS have not demonstrated a significant association between the *PPARγ* locus and CD or UC. The nearest locus with documented association in recent GWAS is at 3p21, some distance from *PPARγ* at 3p26 [34]. *PPARγ* C161T (rs3856806) is a silent SNP hence it is assumed to be in linkage disequilibrium with a functional mutation nearby in view of its multiple previously reported associations. However, a Haploview analysis of *PPARγ*HapMap data revealed that rs3856806 is not in any haplotype block across the gene, although it does show modest LD with several SNPs in block 1, including *PPARγ* Pro12Ala (rs1801282). LD between rs3856806 and several distant intronic SNPs in *PPARγ* was also observed but these do not appear to be in a position to influence transcription initiation. Hence there does not appear to be any functional mutations within the gene that are in strong LD with rs3856806.

Conclusions

Whilst we have not been able to demonstrate any significant associations between two common *PPARγ* SNPs and CD or UC in an Australian population, the important potential therapeutic role for PPARγ agonists should not be discounted. With important anti-inflammatory, immunoregulatory and possibly tumour suppressive functions, the current role of PPARγ agonists in the therapy of IBD seems likely to be expanded. In addition, it will be exciting to investigate potential differences in the genetic susceptibility of UC and CD between Asian, Chinese and Caucasian populations with large Asian genome wide association studies.

Conflicts of interest

None to declare.

Acknowledgments

Georgia Hume was supported by an NH&MRC postgraduate medical scholarship. Elizabeth Fowler was supported by the Reginald Ferguson Research Fellowship from the University of Queensland. Graham Radford-Smith was supported by a Queensland Government Smart State Clinical Research Fellowship.

References

1. Rousseaux C, Lefebvre B, Dubuquoy L, et al. Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferators-activated receptor-γ. J Exp Med 2005; 201: 1205-1215.
2. Debril MB, Renaud JP, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator-activated receptor gamma. J Mol Med 2001; 79: 30-47.
3. Fajas L, Fruchart JC, Auwerx J. PPARgamma3 mRNA: a distinct PPARgamma mRNA subtype transcribed from an independent promoter. F E B S Lett 1998; 438: 55-60.
4. Dubuquoy L, Dharancy S, Nutten S, Pettersson S, Auwerx J, Desreumaux P. Role of peroxisome proliferator-activated receptor gamma and retinoid X receptor heterodimer in hepato-gastroenterological diseases. Lancet 2002; 360: 1410-1418.
5. Su CG, Wen X, Bailey ST, et al., A novel therapy for colitis utilizing *PPARγ* ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999; 104: 383-389.
6. Desreumaux P, Dubuquoy L, Nutten S, et al. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/ peroxisome-activated receptor γ (*PPARγ*) heterodimer: A basis for new therapeutic strategies. J Exp Med 2001; 193: 827-838.
7. Wachtershauser A, Loitsch SM, Stein J. PPAR-gamma is selectively upregulated in Caco-2 cells by butyrate. Biochem Biophys Res Commun 2000; 272: 380-385.
8. Lewis JD, Lichtenstein GR, Deren JJ, et al. Rosiglitazone for active ulcerative colitis: a randomized placebo-controlled trial. Gastroenterology 2008; 134: 688-695.
9. Liang HL, Ouyang Q. A clinical trial of combined use of rosiglitazone and 5-aminosalicylate for ulcerative colitis. World J Gastroenterol 2008; 14: 114-119.
10. Dubuquoy L, Jansson EA, Deeb S, et al. Impaired expression of peroxisome proliferator-activated receptorg in ulcerative colitis. Gastroenterology 2003; 124: 1265-1276.
11. Desreumaux P, Ernst O, Geboes K, et al. Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. Gastroenterology 1999; 117: 73-81.
12. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998; 391: 79-82
13. Yamazaki K, Takazoe M, Tanaka T, Kazumori T, Nakamura Y. Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease. J Hum Genet 2002; 47: 469-472.
14. Yamazaki K, Onouchi Y, Takazoe M, Kubo M, Nakamura Y, Hata A. Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn's disease in Japanese patients. J Hum Genet 2007; 52: 575-583.
15. Sugawara K, Olson TS, Moskaluk CA, et al. Linkage to peroxisome proliferator-activated receptor-γ in SAMP1/YitFc mice and in human Crohn's disease. Gastroenterology 2005; 128: 351-360.
16. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARg2 associated with decreased receptor activity, lower body mass index and improved insulin sensitvity. Nat Genet 1998; 20: 284-287.
17. Shrestha UK, Karimi O, Crusius JB, et al. Distribution of peroxisome proliferator-activated receptor-gamma polymorphisms in Chinese and Dutch patients with inflammatory bowel disease. Inflamm Bowel Dis 2009; 16: 312-319.
18. Zhang ZF, Yang N, Zhao G, Zhu L, Wang LX. Association between the Pro12Ala polymorphism of peroxisome proliferator-activated receptor gamma 2 and inflammatory bowel disease: a meta-analysis. PLos One 2012; 7:e30551.
19. Atug O, Tahan V, Eren F, et al. Pro12Ala Polymorphism in the peroxisome proliferator- activated receptor-gamma (*PPARγ*) gene

in inflammatory bowel disease. J Gastrointestin Liver Dis 2008; 17: 433-437.
20. Meirhaeghe A, Fajas L, Helbecque N, et al. A genetic polymorphism of the peroxisome proliferators-activated receptor γ gene influences plasma leptin levels in obese humans. Hum Mol Genet 1998; 7: 435-440.
21. Wang XL, Oosterhof J, Duarte N. Peroxisome proliferator-activated receptorg C161T polymorphism and coronary artery diease. Cardiovasc Res 1999; 44: 588-594.
22. Siezen CL, van Leeuwen AI, Kram NR, Luken ME, van Kranen HJ, Kampman E. Colorectal adenoma risk is modified by the interplay between pathways in archidonic acid pathway genes and fish consumption. Carcinogenesis 2005; 26: 449-457.
23. Jiang J, Gajalakshmi V, Wang J, et al. Influence of the C161T but no the Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma on colorectal cancer in an Indian population. Cancer Sci 2005; 96: 507-512.
24. Duerr RH, Barmada MM, Zhang L, et al. Evidence for an inflammatory bowel disease locus on chromosome 3p26: linkage, transmission/disequilibrium and partitioning of linkage. Hum Mol Genet 2002; 11: 2599-2606.
25. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661-678.
26. Hume GE, Fowler EV, Doecke J, et al. Novel NOD2 haplotype strengthens the association between TLR4 Asp299gly and Crohn's disease in an Australian population. Inflamm Bowel Dis 2008; 14: 585-590.
27. Yamazaki K, McGovern D, Ragoussis J, et al. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. Hum Mol Genet 2005; 14: 3499-3506.
28. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263-265.
29. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004; 118: 229-241.
30. Asano K, Matsushita T, Umeno J, et al. A genome-wide association study identifies three new susceptibility loci for ulcerative colitis in the Japanese population. Nat Genet 2009; 41: 1325-1329.
31. Tysk C, Lindberg E, Jarnerot G, Flodérus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins A study of heritability and the influence of smoking. Gut 1988; 29: 990-996.
32. Katayama K, Wada K, Nakajima A, et al. A Novel PPAR gamma gene therapy to control inflammation associated with inflammatory bowel disease in a murine model. Gastroenterology 2003; 124: 1315-1324.
33. Zhou X, Kong N, Zou H, et al. Therapeutic potential of TGF-B-induced CD4+Foxp3+ regulatory T cells in autoimmune diseases. Autoimmunity 2011; 44: 43-50.
34. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 2008; 40: 955-962.