

Influence of Peroxisome Proliferator-activated Receptor (PPAR) γ Plo12Ala Polymorphism as a Shared Risk Marker for Both Gastric Cancer and Impaired Fasting Glucose (IFG) in Japanese

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Abstract Activation of peroxisome proliferator-activated receptor γ (PPAR γ) has been shown to inhibit the proliferation of gastric cancer cells. A common polymorphism at codon 12 of this gene (Pro12Ala) has been shown to confer protection against diabetes and colorectal cancer. We investigated the influence of PPAR γ gene Plo12Ala polymorphism on the risk of gastric cancer and on the severity of *Helicobacter pylori*-induced gastritis as well as impaired fasting glucose (IFG) in Japanese. About 215 patients with gastric cancer (GC) and 201 patients without GC enrolled in this study. Plo12Ala polymorphism of PPAR γ was investigated by PCR-RFLP in all of the subjects. The gastritis score of noncancerous antral mucosa was calculated by the updated Sydney system. The diagnosis of IFG was based on repeated evidence of serum fasting glucose (SFG) concentration of greater than or equal to 110 mg/dl. The Plo12Ala genotype of PPAR γ showed a significantly higher frequency in GC patients than in controls (OR = 2.43; 95%CI = 1.04–5.67). In contrast, the Plo12Ala genotype held a lower risk of IFG (OR = 0.33; 95%CI = 0.13–0.83). The same genotype was associated with an increased risk of non-cardiac gastric cancer (OR = 2.39; 95%CI = 1.02–5.65), lower third gastric cancer (OR = 3.56; 95%CI = 1.31–9.71), advanced cancer (OR = 2.93;

95%CI = 1.13–7.58), and Lauren's intestinal cancer (OR = 2.94; 95%CI = 1.13–7.66). Among 151 gastric cancer subjects, the atrophy and metaplasia scores of the antral mucosa adjacent to cancer showed a tendency to be higher in those with the 12Ala allele. Our study suggests that the PPAR γ Pro12Ala polymorphism may be a shared risk marker of both IFG and gastric cancer in Japanese.

Keywords Gastric cancer · Peroxisome proliferator-activated receptor (PPAR) γ · Polymorphism · Impaired fasting glucose (IFG)

Introduction

Gastric cancer remains a significant worldwide health burden. Although the incidence of and mortality due to non-cardiac gastric cancer have been decreasing over the last few decades, it still remains second only to lung cancer as the leading cause of cancer mortality worldwide [1, 2]. *Helicobacter pylori* infects the human stomach and causes chronic mucosal inflammation. Although, it has been classified as a carcinogen [3], there is marked variation in the extent of gastric inflammation among *H. pylori*-infected patients, and only a small percentage of them actually develop gastric cancer. This suggests that genetic factors may also play an important role in gastric carcinogenesis.

The peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors that are members of the nuclear receptor superfamily [4]. At least three different PPAR subtypes (α , β , and γ) have been described [5]. PPAR γ is highly expressed in adipocytes and is responsible for the regulation of adipocyte differentiation and glucose homeostasis, but it has also been suggested to act as a regulator of cell proliferation and the inflammatory

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response [6]. When activated by specific ligands, PPAR γ forms heterodimers with retinoid X receptor (RXR), which regulate the expression of target genes by binding to the peroxisome proliferator-responsive element (PPRE) [7, 8]. Expression of PPAR γ also has been demonstrated in other tissues, including the colon, stomach, small intestine, liver, and pancreas [9]. Recently, it was reported that PPAR γ activation had an inhibitory effect on the proliferation of gastric cancer cells [10]. Furthermore, it has been demonstrated that PPAR γ activation can inhibit gastric mucosal inflammation and apoptosis induced by *H. pylori* [11]. These findings suggest that PPAR γ may play a significant role in suppressing gastric mucosal inflammation and tumorigenesis by gastric epithelial cells.

Studies on the PPAR γ gene (PPARG) have identified a number of polymorphisms, including one that causes an amino acid substitution, Pro12Ala [12]. This substitution may significantly alter the conformation of PPAR γ protein, and thus may influence its activity. The PPAR γ Ala allele has been reported to show decreased binding to the promoter element and demonstrates weaker transactivation of responsive promoters in vitro [13].

Some authors have reported that the PPAR γ Pro12Ala polymorphism confers protection against diabetes and colorectal cancer [14–18]. Concerning the effect of PPAR γ Pro12Ala polymorphism on gastric carcinogenesis, however, one recent study found that the PPAR γ 12Ala allele increases the risk of gastric cancer in China [19]. In the present study, we investigated the influence of PPAR γ Pro12Ala polymorphism on the risk of gastric cancer as well as diabetes or impaired fasting glucose (IFG) in Japanese. We also investigated its association with various subtypes and clinicopathologic features of gastric cancer. Furthermore, we investigated the effect of this polymorphism on the histologic severity of *H. pylori*-induced gastritis.

Materials and methods

Study population

We studied 416 patients attending the Gastroenterology Division of Fujita Health University Hospital (Aichi, Japan) from January 2005 to January 2006. The 416 patients contained 215 patients with gastric cancer (GC) who had a mean age of 64.7 (29–93) years and a male:female (M:F) ratio of 153:62 and 201 non-cancer patients who had a mean age of 63.1 (30–90) years and a M:F ratio of 122:79. Non-cancer patients underwent endoscopic examination for the complaint of abdominal discomfort and were diagnosed as having gastric ulcer, duodenal ulcer, gastritis or normal appearances. Gastric and duodenal ulcer patients were all selected from *H. pylori*-positive subjects. Meanwhile, the

diagnosis of gastritis was made by endoscopists when endoscopic appearances such as erosion and reddish spots were observed irrespective of *H. pylori* infection status. GC was diagnosed histologically and was classified according to Lauren's classification [20]. Detailed information was obtained about the stage, anatomical location, venous and lymphatic invasion, lymph node metastasis, distant metastasis, and peritoneal dissemination. Patients who had severe systemic disease and had received non-steroidal anti-inflammatory drugs were excluded from this study. The Ethics Committee of Fujita Health University School of Medicine approved the protocol and written informed consent was obtained from all of the subjects.

Assessment of diabetes or impaired fasting glucose (IFG)

The diagnosis of impaired fasting glucose was based on repeated evidence of serum fasting glucose (SFG) concentration of greater than or equal to 110 mg/dl. Meanwhile, the patients who had a SFG concentration of <110 mg/dl were considered as non-IFG.

Histological examination

Biopsy specimens were obtained from the uninvolved mucosa of the gastric antrum adjacent to cancer for assessment of gastritis. The extent of neutrophil infiltration, mononuclear cell infiltration, atrophy, and metaplasia was assessed according to the updated Sydney system [21], with each factor being scored from 0 (normal) to 3 (marked).

Detection of *H. pylori* infection

The *H. pylori* infection status was determined on the basis of histology, culture, the rapid urease test (RUT), and antibodies to *H. pylori*. Infection was diagnosed when at least one of these four tests was positive.

Genotyping

Genomic DNA was extracted from frozen normal gastric biopsy tissues or peripheral blood cells using the standard phenol/chloroform method. Then PPAR γ Pro12Ala polymorphism was determined by the polymerase chain reaction-restriction fragment length polymorphism technique, as described previously [22]. The forward primer was 5'-ctgatgtcttgactcatggg-3', and the reverse primer was 5'-ggaagacaaactacaagagc-3'. PCR was carried out in a

reaction volume of 25 μ l containing 20 μ g of genomic DNA, 1 \times reaction buffer, 0.125 mmol/l deoxynucleotide triphosphates, 10 pmol of each primer, and 0.6 units of Taq polymerase (Toyobo, Osaka, Japan). DNA was denatured at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 53°C for 30 s, and 72°C for 40 s, with final extension at 72°C for 7 min. Then digestion with 5 units of Hga-I (New England Biolabs, Inc., Beverly, MA) was employed to analyze PPAR γ Pro12Ala polymorphism, yielding products of 295 bp (Pro) and 178 + 117 bp (Ala). Digestion was performed overnight at 37°C, the products were separated on a 3% agarose gel, and were stained with ethidium bromide for visualization. Genotypes were determined by two independent investigators who were blinded to patient data.

Statistical analysis

The χ^2 test was used for comparison of PPAR γ genotype frequencies between the GC and control groups. The odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression with adjustment for age, sex, and *H. pylori* infection status. For the comparison of PPAR γ genotype frequencies between the IFG and non-IFG patients, logistic regression with adjustment for age and sex was performed. Differences of gastritis scores between the two PPAR γ genotypes (Ala carriers and Pro/Pro) were examined by the Mann-Whitney U test. A probability value of less than 0.05 was considered statistically significant in all analyses.

Results

Study population

A total of 416 subjects including 215 GC and 201 non-cancer patients participated in this study. The characteristics of the subjects are summarized in Table 1. There were no significant differences between the two groups with respect to the age distribution, but male sex and *H. pylori* infection were significantly more common in the GC group. The main endoscopic diagnoses in the control group were gastric ulcer in 39 patients (19.4%), duodenal ulcer in 16 patients (8%), gastric + duodenal ulcer in four patients (2%), and gastritis in 142 patients (70.6%). SFG concentration was measured in 396 of all 416 patients and 164 patients with IFG who had a mean age of 66.5 (33–93) years and a male:female (M:F) ratio of 112:52, as well as 232 non-IFG patients who had a mean age of 62.4 (29–90) years and a M:F ratio of 146:86 were identified. There were no significant differences between IFG patients and non-IFG

Table 1 Characteristics of subjects

	Gastric cancer (GC) cases	Patients without GC	<i>P</i>
Subjects (<i>n</i>)	215	201	
Sex [male/female (%/%)]	153/62 (71/29)	122/79 (61/39)	0.03 ^a
Mean age \pm SD (years)	64.7 \pm 11.9	63.1 \pm 12.6	0.46 ^b
<i>H. pylori</i> infection positive ratio (%)	85.1	70.6	0.0005 ^a

^a χ^2 test, ^b Mann–Whitney U test

Table 2 Characteristics of subjects

	IFG cases	Patients without IFG	<i>P</i>
Subjects (<i>n</i>)	164	232	
Sex [male/female (%/%)]	112/52 (68/32)	146/86 (63/37)	0.27
Mean age \pm SD (years)	66.5 \pm 11.6	68.9 \pm 10.45	0.001 ^b
GC patients/patients without GC	89/75	115/117	0.36 ^a

^a χ^2 test, ^b Mann–Whitney U test

patients with respect to sex and occurrence of GC, but age distribution was significantly higher in the IFG patients (Table 2).

PPAR γ genotype

PPAR γ Pro12Ala polymorphism was investigated in all 416 subjects. The frequency of Pro12Ala polymorphism in the non-cancer patients did not deviate significantly from that expected under the Hardy–Weinberg equilibrium and also did not show any significant difference from the genotype distribution revealed by other studies performed in Japanese populations ($\chi^2 = 0.08$, $P = 0.77$) [23]. There were no subjects homozygous for the Ala12 allele of the PPAR γ gene. First, we compared the prevalence of Pro12Ala polymorphism between IFG patients and non-IFG patients by logistic regression analysis and found that the Pro12Ala genotype held a lower risk of IFG (age and sex adjusted OR = 0.33; 95%CI = 0.13–0.83) (Table 3). In contrast, comparison of the genotype frequency between the GC and control groups showed that the PPAR γ Pro12Ala genotype was associated with a significantly higher risk of gastric cancer (OR = 2.43; 95%CI = 1.04–5.67) (Table 4). To investigate whether the PPAR γ Ala12 allele influenced the clinicopathologic features of gastric cancer, the tumor location, stage, Lauren’s classification, lymphatic and venous invasion, lymph node metastasis, peritoneal dissemination, and distant metastasis were included in a stratified analysis. Among these

Table 3 Association between PPAR γ polymorphism and risk of IFG

Variables (<i>n</i>)	Genotype		Pro/Pro vs. Pro/Ala	
	Pro/Pro	Pro/Ala	OR (95%CI)	<i>P</i>
Patients without IFG (201)	209	23	Reference	
IFG (215)	158	6	0.33 (0.13–0.83)	0.02

Data are adjusted for sex and age

clinicopathologic features, we found that PPAR γ Pro12Ala polymorphism increased the risk of non-cardiac gastric cancer (OR = 2.39; 95%CI = 1.02–5.65), lower third gastric cancer (OR = 3.56; 95%CI = 1.31–9.71), advanced cancer (OR = 2.93; 95%CI = 1.13–7.58), and Lauren's intestinal cancer (OR = 2.94; 95%CI = 1.13–7.66). We also found that the Ala12 allele tended to increase the risk of venous invasion (OR = 3.12; 95%CI = 0.98–9.93) and lymph node metastasis (OR = 2.38; 95%CI = 0.89–6.40) (Table 5), but there was no association between this allele and peritoneal dissemination or distant metastasis. In the control group, there were no significant genotype differences among the patients with gastric ulcer, duodenal ulcer, and gastritis (data not shown).

Effect of PPAR γ polymorphism on the severity of gastritis in noncancerous gastric mucosa adjacent to cancer

Among all 21 subjects with 12Ala allele and randomly selected age, sex matched 130 subjects with homozygous for the P12 allele, the atrophy and metaplasia scores of the antral mucosa showed a tendency to be higher in those possessing the 12Ala allele (Table 6), but the neutrophil infiltration and mononuclear cell infiltration scores did not show any correlation between the 12Ala carriers and subjects homozygous for the P12 allele.

Discussion

In two Japanese populations, the frequency of the common polymorphism of PPAR γ Ala12 allele was shown to be significantly lower in individuals with type 2 diabetes than in normal subjects [14, 15]. In addition, the same genotype

was also associated with reduced risk of colorectal cancer [17, 18], suggesting that this allele may confer protection against diabetes and colorectal cancer. In our study, we have also shown the frequency of Ala12 allele was significantly lower among IFG patients. This indicates that the Ala12 allele may be associated with lower risk of both diabetes and IFG. Meanwhile, in agreement with a recent study performed in China [19], we found that the Ala12 allele is associated with the risk of gastric cancer in a Japanese population. The Ala12 allele frequency was significantly higher in patients with gastric cancer than in controls. In 1998, Deeb et al. reported that Pro12Ala polymorphism of PPAR γ was associated with reduced transactivation activity [13]. Activation of PPAR γ has been shown to inhibit cell growth and to induce the apoptosis of gastric cancer cells [10]. Although we did not investigate the effect of PPAR γ polymorphism on PPAR γ activity in human gastric epithelial cells, it is possible that the polymorphism might have altered the activity of PPAR γ . Thus, it seems reasonable for the 12Ala allele to be a risk factor for gastric cancer.

PPAR γ 12Ala allele has been shown to be associated with reduced risk of colorectal cancer and diabetes [14–18]. Presence of the Pro12Ala variant polymorphism is reported to be associated with lower body mass index (BMI), improved insulin sensitivity, and a reduced risk for type 2 diabetes [14–16]. Therefore, it is possible that PPAR γ is associated with risk for colorectal cancers through insulin-related mechanisms.

However, in the stomach, experimental studies have actually demonstrated that activation of PPAR gamma inhibited the growth of gastric cancer cells and suppressed the gastric mucosal inflammation [10, 11, 33]. Considering the function of PPAR gamma in the stomach, our result of PPAR gamma 12Ala allele, which is associated with reduced promoter activity, of increasing the risk of gastric cancer and gastric atrophy may suggest that gastric carcinogenesis may have different genetic background to colorectal carcinogenesis in some measure.

Next, we investigated the effect of PPAR γ Pro12Ala polymorphism on the characteristics of gastric cancer by stratified analysis, and found that the Ala12 allele was associated with a higher risk of non-cardiac, or lower third cancer and intestinal cancer. These results may be explained by both the anti-tumor effect [10, 24] and the anti-inflammatory effect [11, 25–27] of PPAR γ . Correa et al. reported

Table 4 Association between PPAR γ polymorphism and risk of gastric cancer

Variables (<i>n</i>)	Genotype		Pro/Pro vs. Pro/Ala	
	Pro/Pro	Pro/Ala	OR (95%CI)	<i>P</i>
Patients without GC (201)	193	8	Reference	
Overall GC (215)	194	21	2.43 (1.04–5.67)	0.04

Data are adjusted for sex, age, and *H. pylori* infection status

Table 5 Association between PPAR γ polymorphism and clinicopathologic features of gastric cancer

Variables (n)	Genotype		Pro/Pro vs. Pro/Ala	
	Pro/Pro	Pro/Ala	OR (95%CI)	P
Patients without GC (201)	193	8	Reference	
<i>Tumor location</i>				
Cardia (6)	5	1	4.97 (0.48–51.40)	0.18
Non-cardia (209)	190	19	2.39 (1.02–5.65)	0.049
Upper third (17)	16	1	2.50 (0.27–23.11)	0.42
Middle third (101)	92	9	2.04 (0.74–5.65)	0.17
Lower third (87)	77	9	3.56 (1.31–9.71)	0.01
<i>Staging</i>				
Early (105)	96	9	2.03 (0.74–5.54)	0.17
Advanced (91)	80	11	2.93 (1.13–7.58)	0.03
<i>Lauren's classification</i>				
Intestinal type (124)	111	13	2.94 (1.13–7.66)	0.03
Diffuse type (90)	82	7	2.21(0.79–6.21)	0.14
<i>Lymphatic invasion</i>				
Positive (97)	88	9	2.21(0.80–6.08)	0.13
Negative (74)	67	7	2.39 (0.82–7.02)	0.11
<i>Venous invasion</i>				
Positive (50)	44	6	3.12 (0.98–9.93)	0.053
Negative (121)	111	10	1.93 (0.72–5.14)	0.19
<i>Lymph node metastasis</i>				
Positive (101)	89	12	2.38 (0.89–6.40)	0.08
Negative (114)	106	8	1.96 (0.72–5.34)	0.19
<i>Peritoneal dissemination</i>				
Positive (33)	29	4	3.19 (0.88–11.58)	0.08
Negative (182)	166	16	2.31(0.96–5.58)	0.06
<i>Distant metastasis</i>				
Positive (19)	17	2	2.86 (0.55–14.82)	0.21
Negative (196)	177	19	2.36 (0.99–5.63)	0.053

All data are adjusted for sex, age, and *H. pylori* infection status

Table 6 Association between genotypes and histologic scores in noncancerous gastric antral mucosa adjacent to cancer

Genotype (n)	Pro/Pro(130)	Pro/Ala(21)	P
Neutrophil infiltration	0.85 \pm 0.46	1.00 \pm 0.32	0.14
Mononuclear cell infiltration	1.65 \pm 0.62	0.9 \pm 0.64	0.1
Atrophy	1.09 \pm 0.76	1.4 \pm 0.82	0.09
Intestinal metaplasia	1.42 \pm 1.03	1.85 \pm 0.46	0.08

Scores shown are mean \pm SD. Mann–Whitney U test

that gastric atrophy and metaplasia following severe inflammation are an especially strong risk factor for developing the intestinal type of gastric cancer [28, 29]. Uemura et al. also reported that severe gastric atrophy, corpus-predominant gastritis, and intestinal metaplasia are strong risk factors for the development of intestinal-type gastric cancer [30]. PPAR γ Pro12Ala polymorphism may influence both the anti-tumor and anti-inflammatory actions of PPAR γ and thus may modify the risk of developing intestinal gastric cancer, which frequently arises from a background of severe atrophic gastritis in the non-cardiac and lower third regions.

We have also showed that the Ala12 allele is associated with the risk of more advanced stage. Similarly, Ala12 allele also tended to be associated with a higher risk of venous invasion and lymph node metastasis, possibly due to anti-angiogenetic and anti-metaplastic effects of PPAR γ . For example, PPAR γ ligands suppress angiogenesis by induction of apoptosis in endothelial cells [31, 32]. Activation of PPAR γ has also been shown to inhibit peritoneal metastasis by gastrointestinal cancer cells [33], while a decrease of PPAR γ activity has been found in some cancers with metastasis [34, 35]. Thus, PPAR γ may play a key role in suppressing the progression and metastasis of gastric

cancer, so that PPAR γ Pro12Ala polymorphism may also influence these complications. With regard to the association between PPAR γ polymorphism and peritoneal dissemination or distant metastasis, we expected that the 12Ala allele would be associated with the risk of these complications. However, no correlation was observed between the 12Ala allele and the risk of such complications, possibly because the number of patients with peritoneal dissemination and distant metastasis in this study was small, suggesting that further investigation is needed in a larger population.

We also showed that the PPAR γ 12Ala allele is associated with the severity of gastric mucosal atrophy and intestinal metaplasia. Among 151 gastric cancer subjects, the atrophy and metaplasia scores of the antral mucosa showed a tendency to be higher in those possessing the 12Ala allele. Gastric mucosal atrophy and intestinal metaplasia are caused by chronic exposure to inflammation induced by *H. pylori* infection. PPAR γ has an anti-inflammatory effect by regulating the expression of various genes associated with inflammation, and such an effect also occurs in persons with *H. pylori*-related gastritis. Solimani et al. reported that PPAR γ activation by administration of ciglitazone led to a dose-dependent reduction in the severity of mucosal inflammation elicited by *H. pylori* LPS [27]. Rajnish et al. also reported that two PPAR γ ligands (15dPGJ₂ and BRL-49653) significantly attenuated *H. pylori*-induced apoptosis, while co-treatment with PPAR γ agonists blocked the ability of *H. pylori* to activate nuclear factor (NF)- κ B and increase the level of IL-8, a target of (NF)- κ B [11]. Because of the important role that PPAR γ plays with respect to the inflammatory response in *H. pylori*-induced gastritis, PPAR γ Pro12Ala polymorphism, associated with PPAR γ activity, may also be important for determining the severity of gastric mucosal atrophy. However, we did not find any association between PPAR γ Pro12Ala polymorphism and acute or chronic inflammation. Progression of atrophic gastritis is terminated by the development of extensive intestinal metaplasia, so patients with severe atrophic gastritis and intestinal metaplasia often have mild, but not severe inflammation. However, it should be noted that they had had long-term prior exposure to severe mucosal inflammation.

Regarding the histological differences in gastritis, it has been suggested that patients with gastric ulcer have more severe gastric mucosal atrophy and have increased risk of gastric cancer compared to those with duodenal ulcer [29, 36, 37].

But in this study, frequencies of PPAR γ genotypes were not significantly different among patients with gastric ulcer, duodenal ulcer, and gastritis. In addition, when patients with peptic ulcer were included in non-cancer patients, the frequency of PPAR γ 12Ala polymorphism

over all non-cancer patients did not deviate from the frequency of healthy controls reported in previous studies. So we considered the group of patients was appropriate as control subjects in this study.

Although we showed that the PPAR γ 12Ala allele held a higher risk of gastric mucosal atrophy, which supports the result of suppressive effect of PPAR γ against *H. pylori*-induced gastric inflammation in vitro, previous studies have also demonstrated the direct suppressive effect of PPAR γ against gastric cancer cells and their metastasis. Our data suggest that PPAR gamma polymorphism may be an important factor, which determines the risk of developing gastric cancer by diverse mechanisms, not only by increasing the more severe gastric mucosal atrophy. In this study, we assessed the histological severity of gastric mucosal atrophy and intestinal metaplasia only in the antrum. It is well known that the serum pepsinogen (PG) I/II ratio also reflects the severity of gastric mucosal atrophy [38]; future study will be needed to confirm the association of PPAR γ genotype and gastric mucosal atrophy using such markers.

In conclusion, the present study demonstrated that PPAR γ Pro12Ala polymorphism was associated with reduced risk of IFG, but in contrast, the same genotype was also associated with more severe gastric mucosal atrophy, with intestinal metaplasia, and with an increased risk of gastric cancer, especially the intestinal type, non-cardiac cancer, lower third cancer, and advanced cancer, venous invasion, and lymph node metastasis, suggesting that the PPAR γ Pro12Ala polymorphism may be a shared risk factor for both IFG and gastric cancer. However, we only investigated PPAR γ polymorphism in a limited region of Japan. Since the PPAR γ gene polymorphism shows variations in different ethnic groups [39], further studies will be needed in a larger and ethnically diverse population to confirm the influence of this gene on gastric carcinogenesis as well as developing IFG.

References

1. Murray CJ, Lopez AD (1997) Mortality by cause for eight regions of the world: global burden of disease study. *Lancet* 349:1269–1976
2. Parkin DM, Bray F, Ferlay J, Pisani P (2001) Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 94:153–156
3. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Lyon (1994) Schistosomes, liver flukes, *Helicobacter pylori*. *IARC Monogr Eval Carcinog Risks Hum* 61:1–241
4. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM (1995) The nuclear receptor superfamily: the second decade. *Cell* 83:835–839
5. Kliewer SA, Willson TM (1998) The nuclear receptor PPAR-gamma—bigger than fat. *Curr Opin Genet Dev* 8:576–581
6. Corton JC, Anderson SP, Stauber A (2000) Central role of peroxisome proliferator-activated receptors in the actions of

- peroxisome proliferators. *Annu Rev Pharmacol Toxicol* 40:491–518
7. Kliewer SA, Lehmann JM, Willson TM (1999) Orphan nuclear receptors: shifting endocrinology into reverse. *Science* 284:757–760
 8. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 68:879–887
 9. Auwerx J (2002) Nuclear receptors. I. PPAR gamma in the gastrointestinal tract: gain or pain? *Am J Physiol Gastrointest Liver Physiol* 282:G581–585
 10. Takahashi N, Okumura T, Motomura W, Fujimoto Y, Kawabata I, Kohgo Y (1999) Activation of PPARgamma inhibits cell growth and induces apoptosis in human gastric cancer cells. *FEBS Lett* 455:135–139
 11. Gupta RA, Polk DB, Krishna U, Israel DA, Yan F, DuBois RN, Peek RM Jr (2001) Activation of peroxisome proliferator-activated receptor gamma suppresses nuclear factor kappa B-mediated apoptosis induced by *Helicobacter pylori* in gastric epithelial cells. *J Biol Chem* 276:31059–31066
 12. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR (1997) Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 241:270–274
 13. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kusisto J, Laakso M, Fujimoto W, Auwerx J (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284–287
 14. Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, Takeda J, Inoue I, Seino Y, Yasuda K, Hanafusa T, Yamagata K, Awata T, Kadowaki T, Hara K, Yamada N, Gotoda T, Iwasaki N, Iwamoto Y, Sanke T, Nanjo K, Oka Y, Matsutani A, Maeda E, Kasuga M (2000) The Pro12 → Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes* 50:891–894
 15. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, Kadowaki T (2000) The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 271:212–216
 16. Altschuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES (2000) The common PPAR γ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80
 17. Landi S, Moreno V, Gioia-Patricola L (2003) Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor α , NFkB1, and peroxisome proliferator-activated receptor γ with colorectal cancer. *Cancer Res* 63:3560–3566
 18. Murtaugh MA, Ma KN, Caan BJ, Sweeney C, Wolff R, Samowitz WS, Potter JD, Slaterry ML (2005) Interactions of peroxisome proliferator-activated receptor {gamma} and diet in etiology of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 14:1224–1229
 19. Liao SY, Zeng ZR, Leung WK, Zhou SZ, Chen B, Sung JJ, Hu PJ (2006) Peroxisome proliferator-activated receptor-gamma Pro12Ala polymorphism, *Helicobacter pylori* infection and non-cardia gastric carcinoma in Chinese. *Aliment Pharmacol Ther* 23:289–294
 20. Lauren P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 64:31–49
 21. Dixon MF, Genta RM, Yardley JH, Correa P (1996) Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 20:1161–1181
 22. Hara M, Alcoser SY, Qaadir A, Beiswenger KK, Cox NJ, Ehrmann DA (2002) Insulin resistance is attenuated in women with polycystic ovary syndrome with the Pro(12)Ala polymorphism in the PPARgamma gene. *J Clin Endocrinol Metab* 87:772–775
 23. Mori Y, Kim-Motoyama H, Katakura T, Yasuda K, Kadowaki H, Beamer BA, Shuldiner AR, Akanuma Y, Yazaki Y, Kadowaki T (1998) Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor γ 2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men. *Biochem Biophys Res Commun* 252:195–198
 24. Lu J, Imamura K, Nomura S, Mafune K, Nakajima A, Kadowaki T, Kubota N, Terauchi Y, Ishii G, Ochiai A, Esumi H, Kaminishi M (2005) Chemopreventive effect of peroxisome proliferator-activated receptor gamma on gastric carcinogenesis in mice. *Cancer Res* 65:4769–4774
 25. Jiang C, Ting AT, Seed B (1998) PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 391:82–86
 26. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391:79–82
 27. Slomiany BL, Slomiany A (2002) Suppression of gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide by peroxisome proliferator-activated receptor gamma activation. *IUBMB Life* 53:303–308
 28. Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M (1975) A model for gastric cancer epidemiology. *Lancet* 12:58–60
 29. Correa P (1995) *Helicobacter pylori* and gastric carcinogenesis. *Am J Surg Pathol Suppl* 1:S37–43
 30. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ (2001) *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 345:784–789
 31. Gralinski MR, Rowse PE, Breider MA (1998) Effects of troglitazone and pioglitazone on cytokine-mediated endothelial cell proliferation in vitro. *J Cardiovasc Pharmacol* 31:909–913
 32. Xin X, Yang S, Kowalski J, Gerritsen ME (1999) Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis in vitro and in vivo. *J Biol Chem* 274:9116–9121
 33. Sasaki T, Fujii K, Yoshida K, Shimura H, Sasahira T, Ohmori H, Kuniyasu H (2006) Peritoneal metastasis inhibition by linoleic acid with activation of PPARgamma in human gastrointestinal cancer cells. *Virchows Arch* 448:422–427
 34. Badawi AF, Eldeen MB, Liu Y, Ross EA, Badr MZ (2004) Inhibition of rat mammary gland carcinogenesis by simultaneous targeting of cyclooxygenase-2 and peroxisome proliferator-activated receptor gamma. *Cancer Res* 64:1181–1189
 35. Terashita Y, Sasaki H, Haruki N, Nishiwaki T, Ishiguro H, Shibata Y, Kudo J, Konishi S, Kato J, Koyama H, Kimura M, Sato A, Shinoda N, Kuwabara Y, Fujii Y (2002) Decreased peroxisome proliferator-activated receptor gamma gene expression is correlated with poor prognosis in patients with esophageal cancer. *Jpn J Clin Oncol* 32:238–243
 36. El-Omar EM, Penman ID, Ardill JE, Chittajallu RS, Howie C, McColl KE (1995) *Helicobacter pylori* infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology* 109:681–691

37. Lee S, Iida M, Yao T, Shindo S, Nose Y, Akazawa K, Okabe H, Fujishima M (1990) Risk of gastric cancer in patients with non-surgically treated peptic ulcer. *Scand J Gastroenterol* 25: 1223–1226
38. Kekki M, Samloff IM, Varis K, Ihamaki T (1991) Serum pepsinogen I and serum gastrin in the screening of severe atrophic corpus gastritis. *Scand J Gastroenterol* 186 Suppl:109–116
39. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR (1997) Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 241:270–274