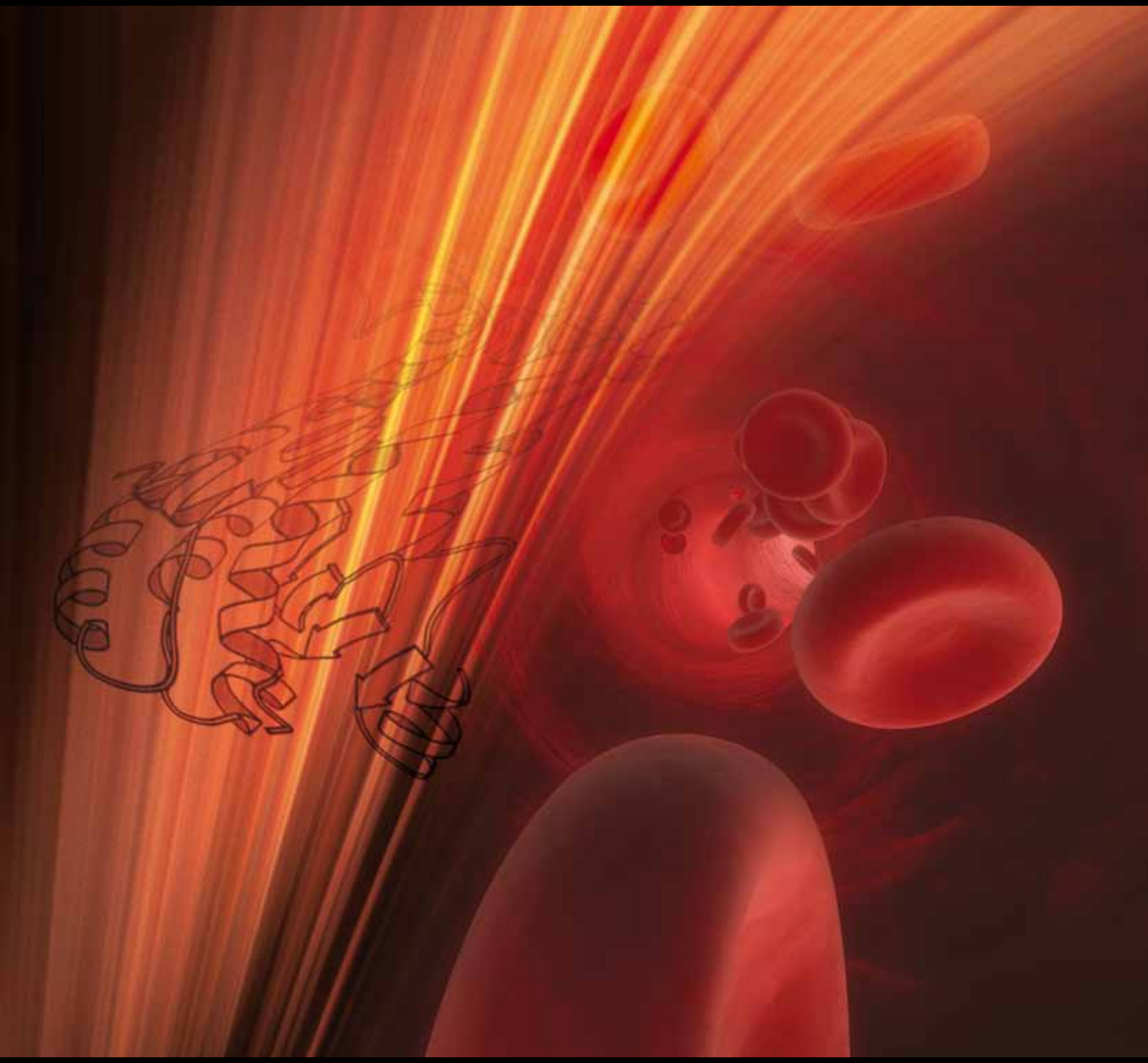
PPAR Research

Genetic Variation of PPARs

Guest Editors: Mostafa Z. Badr, Stefan Wieczorek, and Marie-Claude Vohl



Genetic Variation of PPARs

Genetic Variation of PPARs

Guest Editors: Mostafa Z. Badr, Stefan Wieczorek, and Marie-Claude Vohl

Contents

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 189091, 1 page
doi:10.1155/2009/189091

Editorial

Genetic Variation of PPARs

Marie-Claude Vohl,[1] Mostafa Badr,[2] and Stefan Wieczorek[3]

[1] *Lipid Research Center, CHUL Research Center, Laval University, Quebec City, Canada G1V 4G2*
[2] *Division of Pharmacology, School of Pharmacy, University of Missouri-Kansas City, MO 64108, USA*
[3] *Human Genetics Department, Ruhr-University Bochum, 44801 Bochum, Germany*

Correspondence should be addressed to Marie-Claude Vohl, marie-claude.vohl@crchul.ulaval.ca

Received 15 October 2009; Accepted 15 October 2009

Welcome to this special issue of PPAR Research dedicated to the "Genetic Variation of PPARs." Since PPARs are nuclear transcription factors regulating multiple genes involved in energy production, glucose and lipid metabolism, polymorphisms in these receptors may influence the pathology of numerous diseases including obesity, diabetes, atherosclerosis, inflammation and cancer.

The first section of this special issue of PPAR Research contains a series of four original research articles followed by a review article examining the impact of *PPAR* gene polymorphisms on various metabolic diseases. First is an article by Deeb and Brunzell describing the impact of the Gly482Ser polymorphism in the PPARG coactivator-1 alpha (*PPARGC1A*) on weight gain in a diabetic population. A second article by Dallongeville and coworkers examines the association of *PPARG* gene polymorphisms with coronary heart disease. Third, Wieczorek's research group investigates the consequences of polymorphisms in *RXRB*, *PPARA*, and *PPARG* on Wegener's Granulomatosis. Fourth, a study by Ereqat et al. presents the results of an investigation of the impact of the *PPARG* Pro12Ala polymorphism on the metabolic and clinical characteristics in Palestinian type 2 diabetic patients. Finally this section ends with a review by Weimin He on the influence of the *PPARG* Pro12Ala polymorphism on insulin sensitivity, as well as other diseases including cancer, polycystic ovary syndrome, Alzheimer disease, and aging.

We are also pleased that this special issue contains two articles that describe the functional effects of the *PPAR* gene polymorphisms. First, Rudkowska and co-researchers describe the differences in transcriptional activation observed in two allelic variants of *PPARA* (L162V) after omega-3 fatty acids treatment. Second, McCleelland et al. discern the regulation of translational efficiency by disparate 5′ UTRs of *PPARG* splice variants. These two articles lead to a more complete understanding of the role and functional repercussions of various *PPAR* gene polymorphisms in the prevention and treatment of diseases.

In conclusion, while the influence and impact of PPAR polymorphisms on health and disease is still mostly uncertain, recent evidence suggests that these genetic variations play an important role in the initiation/progression of disease as well as in the efficacy of specific treatments in particular individuals/populations. We are fortunate to have received contributions from such well-renowned experts in the field, and hope that you will find that this special issue of PPAR Research produces greater interest in this critical and evolving field of research.

Marie-Claude Vohl
Mostafa Badr
Stefan Wieczorek

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 649286, 7 pages
doi:10.1155/2009/649286

Research Article

The Role of the PGC1α Gly482Ser Polymorphism in Weight Gain due to Intensive Diabetes Therapy

Samir S. Deeb[1] and John D. Brunzell[2]

[1] *Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, WA 98195-7720, USA*
[2] *Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine, University of Washington, Seattle, WA 98195-7720, USA*

Correspondence should be addressed to Samir S. Deeb, sdeeb@u.washington.edu

Received 13 December 2008; Accepted 14 January 2009

Recommended by Marie-Claude Vohl

The Diabetes Control and Complications Trial (DCCT) involved intensive diabetes therapy of subjects with type 1 diabetes mellitus (T1DM) for an average period of 6.5 years. A subset of these subjects gained excessive weight. We tested for association of polymorphisms in 8 candidate genes with the above trait. We found the Gly482Ser polymorphism in the peroxisome proliferator-activated receptor γ coactivator-1α (PGC1α) to be significantly associated with weight gain in males ($P = .0045$) but not in females. The Ser allele was associated with greater weight gain than the Gly allele ($P = .005$). Subjects with a family history of type 2 diabetes mellitus (T2DM) were more common among those who gained excessive weight. We conclude that T2DM and the Gly482Ser polymorphism in PGC1α contribute to the effect of intensive diabetes therapy on weight gain in males with T1DM.

1. Introduction

The major complications of T1DM are microvascular disease leading to visual impairment and nephropathy, as well as premature cardiovascular disease [1]. The DCCT was designed as a randomized prospective trial to evaluate the benefits of intensive diabetes therapy compared with conventional therapy [2]. After a mean of 6.5 years of intensive diabetes therapy there was a reduction in retinopathy, nephropathy, and neuropathy. Two negative outcomes of intensive diabetes therapy were a threefold increase of episodes of severe hypoglycemia [2] and marked weight gain in a subset of individuals [3]. However, it was shown that the increase in incidence of severe hypoglycemia did not account for excessive weight gain [4]. The top quartile (Q4) of increase in body mass index (BMI) in the intensive therapy had a mean weight gain of 17 kg and their average BMI increased from 24 to 31 [3]. Concomitant with their excess weight gain, they developed components of the metabolic syndrome, including central obesity and insulin resistance.

The aim of this study was to identify genetic factors that contribute to excess weight among T1D patients upon intensive insulin therapy. We tested for association of this trait with sequence variants in 8 candidate genes that are implicated in obesity and/or T2DM (Table 1). The DCCT study offers a unique opportunity to find such genes since it is a large and very well characterized prospective study of subjects with T1DM that have been exposed to intensive therapy and experienced well-documented changes in weight. Whereas there are many pathways to central obesity, the weight gain due to intensive diabetes therapy in this well-characterized DCCT cohort, represents a homogeneous phenotype in which to search for central obesity genes.

2. Methods

2.1. Subjects. The DCCT was designed as a randomized prospective trial to evaluate the benefits of intensive diabetes therapy with multiple insulin injections compared with conventional therapy. A total of 1441 subjects, aged 13–39 years at baseline, 50% females and over 95% Caucasian,

Table 1: *Candidate genes and SNPs analyzed for association with excess weight gain with intensive diabetes therapy.* Genotyping was performed using ABI TaqMan assays with the indicated ABI identification numbers (custom, made to order). Minor allele frequencies are for Caucasian populations derived from published data and the SNP database (http://www.ncbi.nlm.nih.gov/SNP/). *PPARG*, peroxisome proliferator activated receptor γ; *PPARGC1A*, peroxisome proliferator-activated receptor γ coactivator-1α; *HSD11B1*, 11β-hydroxysteroid dehydrogenase 1; GR (*NRC31*), glucocorticoid receptor; *GNB3,* G protein β3 subunit; *ADIPOQ*, adiponectin; *NPY*, neuropeptide y, *FTO* (aliases *KIAA1752*, *MGC5149*), fat mass, and obesity-associated protein.

Gene	Chromosomal location	NCBI refSNP number	TaqMan ABI assay ID	Polymorphism	Minor allele frequency
PPARγ2	3p25	rs1801282	1129864	Pro12Ala (exon 1)	0.10
PPARGC1A	4p15.1	rs8192678	Custom 1643184	Gly482Ser T/C (5′ UTR)	0.34
		rs2279525			0.31
11-β HSD1	1q32	rs2884090	2502457	C/T; intron 4	0.21
GR (NRC31)	5q34	rs6188	1046353	G/T; intron 5	0.31
		rs11749561	178285	T/C; intron 3	
GNB3	12p13	rs5443	2184734	C825T Silent/AS	0.30
ADIPOQ	3q27	rs266729	Custom 2412786	C-377G; promoter	0.29
NPY	7p15.1	rs5573	11164468	Ser22Ser	0.44
FTO	16q12.2	rs9939609	Custom 30090620	T to A in 3′ UTR	0.43

were randomized to conventional or intensive therapy and followed for 3.5–9 years (mean 6.5 years). Subjects in the conventional therapy group [5] typically received one or two insulin injections per day and had a quarterly follow-up at their DCCT clinic. Intensive therapy subjects practiced more rigorous diabetes management by taking three or more insulin injections per day or using an insulin infusion pump, self-monitored their blood glucose four or more times per day, and visited their DCCT care providers monthly to achieve HbA_{1c} levels at least one percentage point lower than the conventional therapy group. All enrolled subjects were in good general health.

The 582 (48% females and 52% males) subjects in this study were in the intensive therapy group and were 18 years of age or older at DCCT entry [2]. They had previously been divided into quartiles of change in BMI over the 6.2 years of the DCCT [3]. The top quartile (Q4) of increase in BMI in the intensive therapy had a mean weight gain of 17 kg and their average BMI increased from 24 to 31. Concomitant with their excess weight gain, they developed components of the metabolic syndrome, including central obesity and insulin resistance, which are strong risk factors for T2DM and for familial combined hyperlipidemia [5].

2.2. Selection of Candidate Genes. Previous reports of linkage and association of gene variants with obesity indices and/or T2DM were the primary criteria in prioritizing the candidate genes for this association study. In addition, priority was given to genes that contain common variants with proven functional consequences. Originally, 7 candidate genes were chosen, and more recently the *FTO* (fat and obesity associated protein) gene was added to this study because of the recent findings that polymorphisms in this gene were associated with obesity (see what follows).

The 8 candidate genes and the respective single nucleotide polymorphisms that were tested for association in this study are listed, in order of decreasing priority, in Table 1. We previously showed that the Pro12Ala polymorphism in the peroxisome proliferator-activated receptorγ2 (PPARγ2), a transcription factor that is critical for adipogenesis, was associated with obesity and T2DM [6]. This polymorphism is among a few that have been confirmed to be associated with T2DM [7]. A second good candidate gene (*PPARGCA1*) encodes the PPRγ coactivator-1α (PGC-1α), common variants which, particularly the Gly428Ser substitution, were observed in some studies to be associated with obesity and T2DM [8–10]. PGC-1α controls the expression of a number of genes involved in energy homeostasis [11, 12] that were shown to be coordinately reduced in individuals with insulin resistance and T2DM [13, 14]. Polymorphisms in the glucocorticoid receptor (GR) gene have been reported to be associated with altered glucocorticoid sensitivity and central obesity [15–17]. 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) which plays an important role in determining tissue glucocorticoid levels has also been implicated in features of the metabolic syndrome in both humans and transgenic mice by amplifying the effects of glucocorticoid action [18–20]. The gene encoding the G protein β3 subunit (*GNB3*) was considered as a good candidate for the metabolic syndrome and T2DM because it contains a common polymorphism (C825T) that was observed to be associated in some studies with development of obesity in young individuals across different ethnic groups, body fat distribution and hypertension. Furthermore, the T allele causes alternate splicing with functional consequences [21–23]. A genome wide scan for the metabolic syndrome detected significant linkage to chromosomal region 3q27 where the *APM1* gene encoding adiponectin is located [24]. Furthermore, genetic variation in *APM1* was observed to be associated with obesity, insulin resistance [25], and T2DM [26]. Neuropeptide Y (NPY) is a major regulator of food consumption and energy homeostasis. Increased NPY signaling in the hypothalamus leads to obesity and its complications such as T2DM and cardiovascular disease. Polymorphisms in the *FTO* gene were

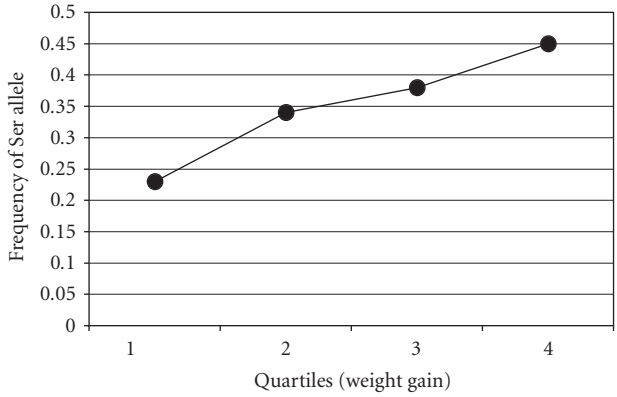


Figure 1: Association of the *PPARGC1A* Ser^{482} allele with weight gain in all quartiles of males with T1DM on intensive diabetes therapy. The frequency of the Ser allele in Q1 (N = 126) was 0.23; Q2 (N = 172) was 0.34; Q3 (N = 158) was 0.38; Q4 (N = 102) was 0.45. The Ser allele was significantly associated with a trend for higher weight gain with a P-value of .004 (Chi-square with 3 degrees of freedom), and a P-value of .0004 (trend, Cochran-Armitage).

shown by a genome-wide association analysis [27] to be associated with BMI and with predisposition to childhood and adult obesity (reviewed in [28]).

2.3. Genotyping. DNA samples from the subjects were prepared from peripheral white blood cells at the Diabetes/Endocrine Research Center at the University of Washington, USA. Single nucleotide polymorphism (SNP) genotyping was performed by real-time PCR amplification on an Applied Biosystems (ABI) 3500 apparatus using ABI TaqMan assays purchased from the manufacturer. The ABI assay identification numbers are given in Table 1.

2.4. Statistical Analysis. The frequency of alleles and genotypes were compared in Q1 and Q4 by the chi-square (χ^2) test. Bonferroni corrections to the significance of associations of the PGC1α Gly482Ser polymorphism with weight gain were not made because of our study design that placed *PPAR*γ2 and *PGC1*α as top priority candidate genes (Table 1). All of the genes we tested had already been reported to be associated with either T2DM or obesity.

3. Results

A total of 252 DCCT non-Hispanic white North American subjects (141 females and 111 males) comprising the upper and lower quartiles of weight gain with intensive therapy were initially genotyped, in a double-blinded manner, for a single common SNP in each of 7 candidate genes (Table 1). The subjects were originally stratified into quartiles regardless of gender. Quartile 1 (Q1) had 64 females and 63 males gained the least amount of weight and quartile 4 (Q4) had 85 females and 51 males and gained the highest amount of weight. Allelic and genotypic frequencies were initially compared between Q1 and Q4 regardless of gender. The results of this analysis revealed a significant difference in allelic and genotypic frequencies of the Gly482Ser polymorphism (rs8192678) in *PPARGC1A*. The Gly/Gly genotype frequency was 0.49 and 0.32 in Q1 and Q4, respectively, (P = .028) and the Gly/Ser genotype was 0.42 and 0.58 in Q1 and Q4, respectively, (P = .028). The genotypic distributions were in Hardy-Weinberg equilibrium (P = .25). The frequency of the Ser allele was 0.30 and 0.39 in Q1 and Q4, respectively, (P = .08). The frequencies of alleles and genotypes in all other 6 candidate genes were not significantly different between Q1 and Q4.

Next, we asked whether gender plays a role in the observed association. Interestingly, we found that the Gly482Ser polymorphism in *PPARGC1A* was associated with weight gain on intensive diabetes therapy only in males (Table 2). The frequency of the Ser allele in males of Q1 was 0.23 compared to 0.45 in males of Q4 (P = .005). The Ser allele frequency was almost identical in females of Q1 and Q4. As expected, the frequencies of genotypes in males were also significantly different between the two quartiles (Table 2). No other covariates were tested for association of the polymorphisms with weight gain.

In an attempt to validate the above association, we genotyped male subjects belonging to Q2 and Q3 for the Gly482Ser polymorphism and examined the association with the extent of weight gain on intensive diabetes therapy in all quartiles. The results are plotted in Figure 1. The frequency of the Ser allele in Q1 (N = 126) was 0.23; Q2 (N = 172) was 0.34; Q3 (N = 158) was 0.38; Q4 (N = 102) was 0.45. As observed with Q1 and Q4, the Ser allele was significantly associated with a trend for higher weight gain with a P-value of .004 (Chi-square with 3 degrees of freedom), and a P-value of .0004 (trend, Cochran-Armitage).

No significant differences in allele and genotype differences between Q1 and Q4 in either males or females were found for the rest of the candidate genes. The Gly482Ser polymorphism is widespread among various ethnic groups. The frequency of the Ser allele was reported to be 0.34 among Germans [10], 0.30 among Danes [8], 0.34 among European Americans (this study), 0.13 among African Americans (unpublished data from our laboratory), 0.18 among Pima Indians [29], 0.43 among Japanese [9], and 0.39 among Chinese (dbSNP at http://www.ncbi.nlm.nih.gov/SNP/).

Subsequently, we assessed the association of a second polymorphism (rs 2279525) located in the 5′ untranslated region (5′ UTR) of the PGC-1α gene (Figure 2). No association with weight gain was observed in either males or females (Table 2). This polymorphism is approximately 46.6 KB upstream of the Gly482Ser polymorphism [30], and the two polymorphisms are in linkage equilibrium (http://www.hapmap.org/ and our data).

4. Discussion

We found that the common and widespread Gly482Ser polymorphism in PGC1α is significantly associated with excessive weight gain in male but not female subjects with T1DM who had undergone intensive diabetes therapy. The less common Ser allele was associated with a higher level

Table 2: *Genotype and allele frequencies of the variants in candidate genes examined for association with weight gain by DCCT subjects on intensive diabetes therapy. N*, number of subjects; the *P*-values compare genotype and minor allele frequencies between quartile 1 (Q1) and quartile 4 (Q4) of weight gain; NS, statistically not significant. The brackets indicate that heterozygotes and homozygotes for the minor allele were combined for comparisons between quartiles.

Gene/polymorphism	Frequency in females			Frequency in males		
	Q1 (*N*, %)	Q4 (*N*, %)	*P*	Q1 (*N*, %)	Q4 (*N*, %)	*P*
PPARG2						
Pro/Pro	52 (82.5)	62 (78.5)	NS	45 (75.0)	42 (84.2)	NS
Pro/Ala	11 (17.5)	17 (21.5)	NS	15 (25.0)	9 (17.6)	NS
Ala/Ala	0 (0)	0 (0)		0 (0)	0 (0)	NS
Allele frequency	0.09	0.11	NS	0.13	0.09	NS
PPARGC1A						
Gly/Gly	25 (40.3)	30 (38.0)	NS	35 (58.3)	12 (23.5)	.0054
Gly/Ser	[28 (45.2)	43 (54.4)]	NS	[22 (36.7)	32 (62.8)]	.0131
Ser/Ser	9 (14.5)	6 (7.6)]		3 (5.0)	7 (13.7)]	
Frequency of Ser	0.37	0.35	NS	0.23	0.45	.0050
PPARGC1A						
$T/\tilde{T}$	28 (45.2)	35 (44.9)	NS	25 (42.3)	28 (54.9)	NS
$T/\tilde{C}$	[27 (43.5)	38 (48.7)]	NS	[26 (44.1)	19 (37.3)]	NS
C/C	7 (11.3)	5 (6.4)]		8 (13.6)	4 (7.8)]	
Frequency of *C*	0.33	0.30	NS	0.35	0.27	NS
HSD11B1						
C/C	41 (66.1)	50 (59.5)	NS	42 (66.7)	32 (62.7)	NS
C/T	[18 (29.0)	28 (33.3)]	NS	[16 (25.4)	17 (33.3)]	NS
T/T	3 (4.9)	6 (7.2)]		5 (7.9)	2 (4.0)]	
Frequency of *T*	0.19	0.23	NS	0.21	0.21	NS
GNB3						
C/C	34 (54.8)	35 (44.3)	NS	30 (50.0)	28 (54.9)	NS
C/T	[23 (37.1)	33 (41.8)]	NS	[23 (38.3)	19 (37.3)]	NS
T/T	5 (8.1)	11 (13.9)]		7 (11.7)	4 (7.8)]	
Frequency of *T*	0.27	0.35	NS	0.31	0.27	NS
NRC31						
G/G	27 (42.2)	41 (52.6)	NS	29 (48.3)	25 (49.0)	NS
G/T	[29 (45.3)	34 (43.6)]	NS	[22 (36.7)	25 (49.0)]	NS
T/T	8 (12.5)	3 (3.8)]		9 (15.0)	1 (2.0)]	
Frequency of *T*	0.35	0.26	NS	0.33	0.27	NS
ADIPOQ						
C/C	32 (52.5)	49 (62.0)	NS	32 (53.3)	30 (58.8)	NS
C/G	[22 (36.1)	24 (30.4)]	NS	[26 (43.4)	17 (33.3)]	NS
G/G	7 (11.4)	6 (7.6)]		2 (3.3)	4 (7.9)]	
Frequency of *G*	0.30	0.23	NS	0.25	0.25	NS
NPY						
G/G	19 (30.6)	21 (26.6)	NS	15 (25.0)	10 (19.6)	NS
G/A	[29 (46.8)	34 (43.0)]	NS	[32 (53.3)	27 (52.9)]	NS
A/A	14 (22.6)	24 (30.4)]		13 (21.7)	14 (27.5)]	
Frequency of *A*	0.46	0.52	NS	0.48	0.54	NS
FTO						
TT	23 (36.0)	29 (35.0)	NS	16 (25.4)	13 (27.1)	NS
TA	32 (50.0)	40 (48.2)	NS	35 (55.6)	25 (52.1)	NS
AA	9 (14.0)	14 (17.0)	NS	12 (19.0)	10 (20.8)	NS
Frequency of *A*	0.39	0.41	NS	0.47	0.47	NS

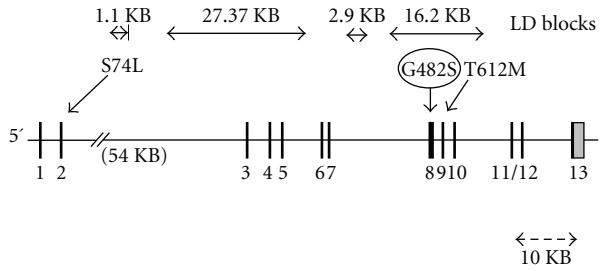


Figure 2: Structure of the *PPARGC1A* gene showing location of linkage disequilibrium (LD) blocks and sequence polymorphisms.

of weight gain. Subjects who gained excessive weight had a higher frequency of T2DM among their parents. Therefore, we hypothesized that the Gly482Ser polymorphism may lead to excessive weight gain in DCCT via its association with the metabolic syndrome and T2DM.

PGC1α is a powerful transcriptional coactivator of several nuclear receptors, including PPARγ, that regulate key metabolic steps in energy homeostasis and glucose metabolism. The level of PGC1α mRNA in skeletal muscle was observed to be lower in individuals with insulin resistance and T2DM [31]. In addition, the expression of PGC1α-responsive genes involved in oxidative phosphorylation is reduced in skeletal muscle of individuals with T2DM, their healthy first degree relatives and even those with impaired glucose tolerance [13, 14]. Importantly, PGC1α mRNA levels in skeletal muscle were lower among carriers of the Ser^{482} allele [31]. Therefore, it is likely that PGC1α with Ser at position 428 is a less efficient coactivator of transcription factors, including those that regulate the *PPARGC1A* gene itself [32]. Therefore, lower PGC1α activity levels may lead to lower levels of glucose and fatty acid oxidation and to fat accumulation [14].

In this study, the Ser^{482} allele predicted excessive weight gain with intensive diabetes therapy in males, but not in females. Two studies have also reported a gender-specific association of the Ser allele with obesity. Ridderstråle et al. reported association of the Ser allele with increased risk of obesity in physically inactive men over 50 years of age, but not in women [33]. However, in another study [10] the association was observed in middle-aged women but not men. The reason for this conflicting result could be attributed to the older age of subjects than those in DCCT, for example, the above middle-aged women could have been postmenopausal. Also, our subjects had T1DM and their weight gain was due specifically to intensive diabetes therapy. The Ser allele was also found to be associated with T2DM in Northern Chinese, especially in males [34]. We do not have an explanation for the gender-specific association. One possibility is that activity levels of PGC1α may be coregulated by estrogens. This is compatible with the observation that the level of muscle PGC1α mRNA is lower in females than males [31]. Therefore, effects of the Gly482Ser polymorphism on weight gain may be attenuated by the action of estrogens.

The following genetic association studies support the hypothesis that the PGC1α Gly482Ser polymorphism affects weight gain via its effect on the metabolic syndrome (which includes insulin resistance and abdominal obesity) and T2DM. First, a meta-analysis showed a modest association of the Ser^{482} allele with the risk for T2DM [35]. These case-control study populations included both males and females (3,718 cases and 4,818 controls) that were, unfortunately, not independently tested for association. Second, a locus on chromosome 4p15.1, where the *PPARGC1A* is located, was found to be associated with abdominal obesity [36] and insulin resistance [37]. Third, the PPARγ2 Pro12Ala and the PGC1α Gly482Ser polymorphisms were associated with conversion from impaired glucose tolerance to T2DM in the STOP-NIDDM trial [38]. The PPARγ2 Pro12Ala polymorphism has been consistently shown to be associated with risk for T2DM [6, 7, 39]. Fourth, The Gly482Ser polymorphism is located in a highly conserved domain that was shown to interact with and coactivate the muscle enhancer factor 2C that activates the GLUT4 gene [40]. Importantly, the Ser^{482} allele was consistently observed to be associated with insulin resistance, T2DM or obesity. This monoallelic association in different populations represents a strong validation for this association.

Haplotype analysis at the *PPARGC1A* gene locus in Caucasians revealed the existence of 4 adjacent multi-SNP linkage disequilibrium (LD) blocks with a D′ of close to 1 (Figure 2) (http://www.hapmap.org/). The Gly482Ser polymorphism lies within the 16.2 KB LD block. This block contains 10 haplotypes based on 8 single-SNP genotypes (http://www.broad.mit.edu/mpg/haploview/). Only one haplotype contains the Ser allele and is the most common one (0.367). The other seven haplotypes have Gly, the most common (0.224) differs from the Ser-containing haplotype at 8 of the 13 SNPs. Therefore, it is possible that variants in LD with the Ser allele could contribute to the observed association. Another polymorphism in exon 9 (Thr612Met) (Figure 2) that lies within the 16.2 KB LD block could also influence the activity of PGC1α [8]. However, its frequency is quite low in the general population (Met allele frequency of 0.03) and, therefore, is unlikely to have a major impact on the observed association with the Ser482 allele.

The polymorphism in the 5′ untranslated region of *PPARGC1A* (Figure 2) was not associated with weight gain in either sex in our study. Since this polymorphism is in LD with those in exon 1, our result is consistent with lack of association between the Ser74Leu polymorphism in exon 2 (Figure 2) and T2DM [8].

No significant association of variants in the other 7 candidate genes was observed. This does not mean that these genes do not contribute to excessive weight gain. The relatively small sample size and the low frequency of some of the polymorphisms may have resulted in low power to detect an association. A more thorough analysis of other variants in these genes is necessary using a larger population sample in order to detect moderate effects on weight gain.

5. Conclusions

The common PGC1α-Gly482Ser polymorphism was significantly associated with excessive weight gain in male, but not female subjects with T1DM who had undergone intensive diabetes therapy. The less common Ser allele was associated with a higher level of weight gain. PGC1α Ser is a less efficient coactivator of transcription factors. Therefore, lower PGC1α activity levels may lead to lower levels of glucose and fatty acid oxidation and to fat accumulation.

Acknowledgments

This study was supported by a National Institutes of Health (NIDDK) Grant no. P01 DK02456 and the Diabetes Endocrinology Research Center National Institutes of Health Grant no. DK 17047. We also thank the DCCT/EDIC investigators, support staff, participants, and their families. We appreciate the technical effort of Li Fu in genotyping the DNA samples.

References

[1] A. S. Krolewski, E. J. Kosinski, J. H. Warram, et al., "Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus," *The American Journal of Cardiology*, vol. 59, no. 8, pp. 750–755, 1987.

[2] The Diabetes Control and Complications Trial Research Group, "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus," *The New England Journal of Medicine*, vol. 329, no. 14, pp. 977–986, 1993.

[3] J. Q. Purnell, J. E. Hokanson, S. M. Marcovina, M. W. Steffes, P. A. Cleary, and J. D. Brunzell, "Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT," *The Journal of the American Medical Association*, vol. 280, no. 2, pp. 140–146, 1998.

[4] J. Q. Purnell, R. K. Dev, M. W. Steffes, et al., "Relationship of family history of type 2 diabetes, hypoglycemia, and autoantibodies to weight gain and lipids with intensive and conventional therapy in the diabetes control and complications trial," *Diabetes*, vol. 52, no. 10, pp. 2623–2629, 2003.

[5] M. C. Carr and J. D. Brunzell, "Abdominal obesity and dyslipidemia in the metabolic syndrome: importance of type 2 diabetes and familial combined hyperlipidemia in coronary artery disease risk," *The Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 6, pp. 2601–2607, 2004.

[6] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[7] D. Altshuler, J. N. Hirschhorn, M. Klannemark, et al., "The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes," *Nature Genetics*, vol. 26, no. 1, pp. 76–80, 2000.

[8] J. Ek, G. Andersen, S. A. Urhammer, et al., "Mutation analysis of peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to type II diabetes mellitus," *Diabetologia*, vol. 44, no. 12, pp. 2220–2226, 2001.

[9] K. Hara, K. Tobe, T. Okada, et al., "A genetic variation in the PGC-1 gene could confer insulin resistance and susceptibility to type II diabetes," *Diabetologia*, vol. 45, no. 5, pp. 740–743, 2002.

[10] H. Esterbauer, H. Oberkofler, V. Linnemayr, et al., "Peroxisome proliferator-activated receptor-γ coactivator-1 gene locus: associations with obesity indices in middle-aged women," *Diabetes*, vol. 51, no. 4, pp. 1281–1286, 2002.

[11] P. Puigserver and B. M. Spiegelman, "Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): transcriptional coactivator and metabolic regulator," *Endocrine Reviews*, vol. 24, no. 1, pp. 78–90, 2003.

[12] A. R. Shuldiner and J. C. McLenithan, "Genes and pathophysiology of type 2 diabetes: more than just the Radle cycle all over again," *The Journal of Clinical Investigation*, vol. 114, no. 10, pp. 1414–1417, 2004.

[13] V. K. Mootha, C. M. Lindgren, K.-F. Eriksson, et al., "PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes," *Nature Genetics*, vol. 34, no. 3, pp. 267–273, 2003.

[14] M. E. Patti, A. J. Butte, K. Cusi, et al., "Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of *PGC1* and *NRF1*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 14, pp. 8466–8471, 2003.

[15] M. G. Dobson, C. P. F. Redfern, N. Unwin, and J. U. Weaver, "The N363S polymorphism of the glucocorticoid receptor: potential contribution to central obesity in men and lack of association with other risk factors for coronary heart disease and diabetes mellitus," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 5, pp. 2270–2274, 2001.

[16] R. Rosmond, Y. C. Chagnon, G. Holm, et al., "A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis," *Obesity Research*, vol. 8, no. 3, pp. 211–218, 2000.

[17] E. F. C. van Rossum and S. W. J. Lamberts, "Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition," *Recent Progress in Hormone Research*, vol. 59, pp. 333–357, 2004.

[18] E. Rask, T. Olsson, S. Soderberg, et al., "Tissue-specific dysregulation of cortisol metabolism in human obesity," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 3, pp. 1418–1421, 2001.

[19] H. Masuzaki, J. Paterson, H. Shinyama, et al., "A transgenic model of visceral obesity and the metabolic syndrome," *Science*, vol. 294, no. 5549, pp. 2166–2170, 2001.

[20] J. R. Seckl and B. R. Walker, "Minireview: 11β-hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action," *Endocrinology*, vol. 142, no. 4, pp. 1371–1376, 2001.

[21] W. Siffert, "G protein β_3 subunit 825T allele, hypertension, obesity, and diabetic nepropathy," *Nephrology Dialysis Transplantation*, vol. 15, no. 9, pp. 1298–1306, 2000.

[22] M. Rydén, G. Faulds, J. Hoffstedt, A. Wennlund, and P. Arner, "Effect of the (C825T) Gβ_3 polymorphism on adrenoceptor-mediated lipolysis in human fat cells," *Diabetes*, vol. 51, no. 5, pp. 1601–1608, 2002.

[23] N. Stefan, M. Stumvoll, F. Machicao, M. Koch, H. U. Häring, and A. Fritsche, "C825T polymorphism of the G protein β_3 subunit is associated with obesity but not with insulin sensitivity," *Obesity Research*, vol. 12, no. 4, pp. 679–683, 2004.

[24] A. H. Kissebah, G. E. Sonnenberg, J. Myklebust, et al., "Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 26, pp. 14478–14483, 2000.

[25] C. Menzaghi, T. Ercolino, R. D. Paola, et al., "A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome," *Diabetes*, vol. 51, no. 7, pp. 2306–2312, 2002.

[26] K. Hara, P. Boutin, Y. Mori, et al., "Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population," *Diabetes*, vol. 51, no. 2, pp. 536–540, 2002.

[27] T. M. Frayling, N. J. Timpson, M. N. Weedon, et al., "A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity," *Science*, vol. 316, no. 5826, pp. 889–894, 2007.

[28] C. Dina, "New insights into the genetics of body weight," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 11, no. 4, pp. 378–384, 2008.

[29] Y. L. Muller, C. Bogardus, O. Pedersen, and L. Baier, "A Gly482Ser missense mutation in the peroxisome proliferator-activated receptor γ coactivator-1 is associated with altered lipid oxidation and early insulin secretion in Pima Indians," *Diabetes*, vol. 52, no. 3, pp. 895–898, 2003.

[30] H. Esterbauer, H. Oberkofler, F. Krempler, and W. Patsch, "Human peroxisome proliferator activated receptor gamma coactivator 1 (*PPARGC1*) gene: cDNA sequence, genomic organization, chromosomal localization, and tissue expression," *Genomics*, vol. 62, no. 1, pp. 98–102, 1999.

[31] C. Ling, P. Poulsen, E. Carlsson, et al., "Multiple environmental and genetic factors influence skeletal muscle *PGC-1α* and *PGC-1β* gene expression in twins," *The Journal of Clinical Investigation*, vol. 114, no. 10, pp. 1518–1526, 2004.

[32] C. Handschin, J. Rhee, J. Lin, P. T. Tarr, and B. M. Spiegelman, "An autoregulatory loop controls peroxisome proliferator-activated receptor γ coactivator 1α expression in muscle," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 12, pp. 7111–7116, 2003.

[33] M. Ridderstråle, L. E. Johansson, L. Rastam, and U. Lindblad, "Increased risk of obesity associated with the variant allele of the PPARGC1A Gly482Ser polymorphism in physically inactive elderly men," *Diabetologia*, vol. 49, no. 3, pp. 496–500, 2006.

[34] L. Sun, Z. Yang, F. Jin, et al., "The Gly482Ser variant of the PPARGC1 gene is associated with type 2 diabetes mellitus in northern Chinese, especially men," *Diabetic Medicine*, vol. 23, no. 10, pp. 1085–1092, 2006.

[35] I. Barroso, J. Luan, M. S. Sandhu, et al., "Meta-analysis of the Gly482Ser variant in *PPARGC1A* in type 2 diabetes and related phenotypes," *Diabetologia*, vol. 49, no. 3, pp. 501–505, 2006.

[36] L. Pérusse, T. Rice, Y. C. Chagnon, et al., "A genome-wide scan for abdominal fat assessed by computed tomography in the Québec Family Study," *Diabetes*, vol. 50, no. 3, pp. 614–621, 2001.

[37] R. E. Pratley, D. B. Thompson, M. Prochazka, et al., "An autosomal genomic scan for loci linked to prediabetic phenotypes in pima indians," *The Journal of Clinical Investigation*, vol. 101, no. 8, pp. 1757–1764, 1998.

[38] L. Andrulionytè, J. Zacharova, J.-L. Chiasson, and M. Laakso, "Common polymorphisms of the *PPAR-γ2* (*Pro12Ala*) and *PGC-1α* (*Gly482Ser*) genes are associated with the conversion from impaired glucose tolerance to type 2 diabetes in the STOP-NIDDM trial," *Diabetologia*, vol. 47, no. 12, pp. 2176–2184, 2004.

[39] L. J. Scott, K. L. Mohlke, L. L. Bonnycastle, et al., "A genome-wide association study of type 2 diabetes in finns detects multiple susceptibility variants," *Science*, vol. 316, no. 5829, pp. 1341–1345, 2007.

[40] L. F. Michael, Z. Wu, R. B. Cheatham, et al., "Restoration of insulin-sensitive glucose transporter ($GLUT_4$) gene expression in muscle cells by the transcriptional coactivator PGC-1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 7, pp. 3820–3825, 2001.

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 543746, 11 pages
doi:10.1155/2009/543746

Research Article

Peroxisome Proliferator-Activated Receptor Gamma Polymorphisms and Coronary Heart Disease

Jean Dallongeville,[1] Carlos Iribarren,[2] Jean Ferrières,[3] Liisa Lyon,[2] Alun Evans,[4] Alan S. Go,[2] Dominique Arveiler,[5] Stephen P. Fortmann,[6] Pierre Ducimetière,[7] Mark A. Hlatky,[8] Philippe Amouyel,[1] Audrey Southwick,[9] Thomas Quertermous,[10] and Aline Meirhaeghe[1]

[1]*Service d'Epidémiologie et de Santé Publique, Institut Pasteur de Lille, Lille; INSERM, U744, Lille; Université Nord de France, Lille; UDSL, 59019 Lille, France*
[2]*Kaiser Permanente Division of Research, Oakland, CA 94612, USA*
[3]*INSERM, U558, Toulouse, Department of Epidemiology, Paul Sabatier-Toulouse Purpan University, 31073 Toulouse, France*
[4]*The Department of Epidemiology and Public Health, Queen's University Belfast, Belfast BT71NN, Northern Ireland, UK*
[5]*Department of Epidemiology and Public Health, EA 3430, University of Strasbourg, Faculty of Medicine, 67085 Strasbourg, France*
[6]*Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA 94305-5705, USA*
[7]*INSERM, U780, 94807 Villejuif, Hôpital Kremlin-Bicêtre, France*
[8]*Department of Health Research and Policy, Stanford University School of Medicine, Stanford, CA 94305, USA*
[9]*Stanford Human Genome Center, Stanford, CA 94304, USA*
[10]*Falk Cardiovascular Research Center, Stanford Falk Cardiovascular Research Building, Stanford, CA 94305-5406, USA*

Correspondence should be addressed to Jean Dallongeville, jean.dallongeville@pasteur-lille.fr

Received 11 June 2009; Accepted 26 August 2009

Recommended by Mostafa Badr

Single nucleotide polymorphisms (SNPs) in the peroxisome proliferator-activated receptor γ (*PPARG*) gene have been associated with cardiovascular risk factors, particularly obesity and diabetes. We assessed the relationship between 4 *PPARG* SNPs (C-681G, C-689T, Pro12Ala, and C1431T) and coronary heart disease (CHD) in the PRIME (249 cases/494 controls, only men) and ADVANCE (1,076 cases/805 controls, men or women) studies. In PRIME, homozygote individuals for the minor allele of the *PPARG* C-689T, Pro12Ala, and C1431T SNPs tended to have a higher risk of CHD than homozygote individuals for the frequent allele (adjusted OR [95% CI] = 3.43 [0.96–12.27], $P = .058$, 3.41 [0.95–12.22], $P = .060$ and 5.10 [0.99–26.37], $P = .050$, resp.). No such association could be detected in ADVANCE. Haplotype distributions were similar in cases and control in both studies. A meta-analysis on the Pro12Ala SNP, based on our data and 11 other published association studies (6,898 CHD cases/11,287 controls), revealed that there was no evidence for a significant association under the dominant model (OR = 0.99 [0.92–1.07], $P = .82$). However, there was a borderline association under the recessive model (OR = 1.29 [0.99–1.67], $P = .06$) that became significant when considering men only (OR = 1.73 [1.20–2.48], $P = .003$). In conclusion, the *PPARG* Ala12Ala genotype might be associated with a higher CHD risk in men but further confirmation studies are needed.

1. Introduction

The peroxisome proliferator-activated receptor γ (PPARG) is a nuclear hormone receptor that dimerizes with the retinoid X receptor (RXR) to regulate target genes involved in adipocyte differentiation and insulin sensitization [1]. Activation of PPARG with thiazolidinediones is used to stimulate insulin sensitivity in the treatment of type 2 diabetes. PPARG also plays a role in macrophage, malignant breast epithelial, colon cancer cell differentiation, and lipid homeostasis [2] and has been shown to control the expression of proinflammatory genes in vascular cell models [3]. This suggests that PPARG may contribute to the pathogenesis of the atherosclerotic plaque physiology at a very early stage.

Prior investigations have analyzed the relationship between common *PPARG* SNPs and various metabolic disorders. The Pro12Ala substitution in the specific exon B of PPARG2 isoform, which contributes to a lower PPARG2

activity in vitro, was associated with a decreased risk of type 2 diabetes [4]. Obese carriers of the minor allele of the *PPARG* C1431T polymorphism in exon 6 presented higher plasma leptin levels than noncarriers [5]. The C-681G and C-689T polymorphisms in the second and third *PPARG* promoter, respectively, were both associated with higher body weight and plasma LDL concentrations [6, 7]. Finally, haplotype analyses showed that a particular combination of these 4 polymorphisms was more frequent in patients with a metabolic syndrome than in control subjects [8]. Altogether, these observations suggest that genetic variability at the *PPARG* locus could affect cardiovascular risk.

Early studies analyzed the association between the *PPARG* Pro12Ala SNP and coronary heart disease (CHD) in cohorts from North America. These investigations yielded inconsistent results [9, 10]. Furthermore, a higher risk of coronary heart disease was reported in obese subjects carrying the Ala12 allele that needed confirmation [10]. Therefore, the goal of the present study was to explore the association between 4 *PPARG* SNPs, including the Pro12Ala SNP, and CHD risk in two independent studies among white subjects. We also performed haplotype analyses to evaluate whether a particular combination of alleles could better explain the effect of *PPARG* genetic variability on CHD risk and assessed whether overweight and obesity could modulate this risk. Finally, we performed a meta-analysis of published studies to date, focusing on the *PPARG* Pro12Ala polymorphism and CHD risk.

2. Methods

2.1. PRIME Study. The PRIME (Prospective Study of Myocardial Infarction) study is a prospective cohort study designed to identify risk factors for CHD [11]. Details on recruitment, baseline examination, and follow-up of the PRIME Study have been previously reported [12]. Overall, 9758 middle-aged men aged 50–59 years and free of CHD at baseline were recruited in Lille, Strasbourg, and Toulouse in France ($n = 7399$) and Belfast in Northern Ireland ($n = 2359$) between 1991 and 1993 and followed up for 5 years for the occurrence of first CHD events including coronary death, nonfatal myocardial infarction, and stable or unstable angina pectoris.

2.1.1. General Characteristics. Subjects who agreed to take part in the study were given a morning appointment and asked to fast for at least 12 hours. A full description of clinical and laboratory measurements has been published elsewhere [11, 12]. Briefly, a health questionnaire self-administered by subjects at their homes was subsequently checked by trained interviewers at the clinic. It covered a broad range of clinical information, including family and personal clinical histories, tobacco consumption, and drug intake. Blood pressure was measured twice in the sitting position with the same automatic device (Spengler SP9). A 12-lead ECG was also recorded. Plasma lipids analyses were centralized (SERLIA INSERM U325, Institut Pasteur de Lille, France). Overweight and obesity were defined as BMI $\geq 25\,kg/m^2$ and BMI $\geq 30\,kg/m^2$, respectively.

2.1.2. Biochemical Measurements. A subset of biochemical measurements was performed on the entire cohort at baseline. Total cholesterol and triglycerides were measured by enzymatic methods using commercial kits in an automatic analyzer (Boehringer, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol was determined after precipitation of Apo-lipoprotein B by an enzymatic method (Boehringer). Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula. Insulin was assayed by competitive radioimmunoassay (Sanofi-Diagnostic Pasteur, France) in cases and matched controls using plasma samples collected at baseline and stored in liquid nitrogen.

During the follow-up, subjects were contacted annually by letter and asked to complete a clinical event questionnaire. For all subjects reporting a possible event, clinical information was sought directly from the hospital or general practitioner records. All details on ECGs, hospital admissions, enzymes, surgical intervention, angioplasty, treatments, and so forth were collected. Death certificates were checked for supporting clinical and postmortem information on cause of death. Whenever possible, circumstances of death were obtained from the practitioner or the family. A Medical Committee comprising one member from each PRIME Center and the Coordinating Center, and three cardiologists (two from France and one from the UK), was established to provide an independent validation of coronary events. A description of the coronary end point definitions has been published recently [12]. The five-year follow-up was completed in 98,6% of the French participants and 99,2% of the Northern-Irish participants.

A nested case-control study within the PRIME prospective cohort study was mounted using the baseline plasma samples of 335 study participants who subsequently developed a future coronary ischemic event during follow-up and of 670 matched controls (2 controls per case). Matched controls were study participants recruited in the same center, on the same day ($\pm$ 3 days), of the same age ($\pm$ 3 years) as the case and free of CHD at the time of the ischemic event of the case. Subjects with incomplete data were excluded. If a case was excluded, his two matched controls were also excluded, and if the two matched controls for a case were excluded, the corresponding case was also excluded. A total of 249 cases and 494 controls were used in the present study.

2.2. The ADVANCE Study. The Atherosclerotic Disease, VAscular FuNction, and genetiC Epidemiology (ADVANCE) study recruited, between October 2001 and December 2003, a total of 3,179 subjects from multiple race/ethnic background into 5 cohorts: a cohort of subjects with clinically significant CAD at a young age ($\leq$45 years for males, $\leq$55 years for females), a cohort of subjects with incident stable angina at an older age, a cohort of subjects with incident acute myocardial infarction (AMI) at an older age, a cohort of young subjects with no history of CAD, and a cohort of

Table 1: Baseline characteristics of subjects with incident CHD (case) and CHD-free (control) subjects in PRIME and ADVANCE.

	PRIME Men			ADVANCE Men			ADVANCE Women		
	Controls	Cases	P^*	Controls	Cases	P^*	Controls	Cases	P^*
N	494	249		433	706		372	370	
Age (y)	55.1 ± 2.8	55.3 ± 3.0	.46	65.8 ± 3.3	61.5 ± 7.9	<.0001	61.5 ± 6.9	60.0 ± 8.8	.11
BMI (kg/m^2)	26.7 ± 3.5	27.1 ± 3.4	.11	28.3 ± 4.4	29.1 ± 4.8	.005	27.4 ± 6.3	29.6 ± 7.2	<.0001
Waist girth (cm)	93.6 ± 10.1	95.0 ± 10.2	.067	99.4 ± 12.6	100.0 ± 12.0	.46	83.8 ± 13.6	90.4 ± 15.9	<.0001
Years at school (y)	11.26 ± 5	10.9 ± 3	.36	NA	NA		NA	NA	
Physically active (%)	19.6	19.6	.99	62.6	53.8	.004	63.2	45.7	<.0001
Current smokers (%)	30.6	19.7	.0019	6.8	8.4	.36	8.4	13.0	.04
Alcohol consumption (g/week)	236 ± 310	234 ± 323	.88	50 ± 140	20 ± 70	<.0001	20 ± 70	0 ± 30	<.0001
History of hypertension (%)	17.6	30.1	.0001	51.5	78.8	<.0001	41.7	79.2	<.0001
History of dyslipidemia (%)	28.5	34.9	.06	25.4	28.6	.46	11.6	26.2	<.0001
History of diabetes (%)	4.9	8.8	.036	15.5	23.0	<.0001	5.1	21.4	<.0001
Systolic BP (mm Hg)	13 ± 19	141 ± 2	<.0001	131 ± 16	120 ± 17	<.0001	124 ± 19	121 ± 20	.08
Diastolic BP (mm Hg)	84 ± 13	87 ± 12	.0008	75 ± 8	71 ± 9	<.0001	71 ± 9	69 ± 9	.0004
Total cholesterol (mg/dL)	225 ± 37	234 ± 39	.0008	202 ± 35	NA	—	210 ± 36	NA	—
LDL-cholesterol (mg/dL)	146 ± 34	157 ± 33	.0001	124 ± 30	NA	—	124 ± 30	NA	—
HDL-cholesterol (mg/dL)	47 ± 12	43 ± 11	<.0001	49 ± 14	NA	—	62 ± 17	NA	—

Data are expressed as means ± SD or percentages. $^*T$-test for continuous variables and Chi-square test for categorical variables. NA: Not available. BP: blood pressure.

subjects aged 60 to 72 with no history of CAD, ischemic stroke, or peripheral arterial disease (PAD). Eligible subjects were identified using the Kaiser Permanente of Northern California (KPNC) electronic databases and those who agreed to participate were interviewed and examined at one of several clinics in the San Francisco Bay Area. A sixth cohort of young subjects with no history of CAD included 479 participants in the Coronary Artery Risk Development in Young Adults (CARDIA) Study [13] originally recruited through KPNC and attending the study's year 15 examination cycle in 2000-2001. A detailed description of the source population for all cohorts has been published elsewhere [14, 15]. The design of the ADVANCE study allowed for several case control comparisons. In this study, we included all white subjects with symptomatic early onset CAD (young cases), older subjects with either AMI or angina as first presentation of CHD (older cases), young controls, and older controls. This resulted in 1 076 CHD cases (706 in men, 370 in women) and 805 controls (433 male, 372 female).

The ADVANCE study was approved by the Institutional Review Board at both Stanford University and Kaiser Permanente of Northern California (KPNC). Details of the methodology for risk factor assessment can be found elsewhere [14, 15].

2.3. Genotyping. The genotyping method in PRIME has been described previously [8]. The genotyping success rate was above 94% for each SNP. Genotyping in ADVANCE was performed at the Stanford Human Genome Center using the ABI 7900 TaqMan platform [16, 17]. The "no call rate" for genotypes was only 0.35% and the reproducibility was 99.9% in random samples with repeat genotypes.

2.4. Statistical Analyses. For ADVANCE, we used unconditional logistic regression because cases and controls were not individually matched. For PRIME, we used conditional logistic regression analysis to compare the distribution of genotypes between cases and controls and to estimate the odds ratio (OR) of CHD. The analyses were adjusted for age, educational level, alcohol consumption, physical activity, smoking status, history of diabetes, history of hypertension, and history of dyslipidemia. Additional analyses were performed to test the interaction between overweight or obesity and genotype by adding a cross-product term to the logistic regression model. We used general regression models and chi-square test to compare the clinical and biological characteristics of the subjects according to genotype in control subjects. Triglyceride values were log transformed for analyses. All analyses were performed using SAS software 8.2 version (SAS Institute, Cary, USA).

2.4.1. Haplotype Analyses. Presence of linkage disequilibrium between the loci was tested using a log-likelihood-ratio test [18] and the degree of disequilibrium was expressed in terms of normalized difference $D' = D/D_{\max}$ or $D/D_{\min}$ [19]. Haplotype frequencies were estimated using a stochastic version of the expectation-maximization algorithm as implemented in Thesias software [20, 21]. Differences in haplotype frequencies between cases and their respective controls were examined using a log-likelihood ratio statistic

Table 2: *PPARG* genotype distributions among white CHD cases and CHD-free controls and ORs [95% CI] of CHD.

(a)

	PRIME Men						
				Model 1		Model 2	
	Controls	Cases	*P**	OR [95% CI]	*P*	OR [95% CI]	*P*
C-681G (n)	484	243					
CC, n (%)	286 (59.1)	146 (60.1)		reference		reference	
CG, n (%)	174 (35.9)	79 (32.5)	.32	0.88 [0.64–1.22]	.44	0.90 [0.64–1.26]	.53
GG, n (%)	24 (5.0)	18 (7.4)		1.54 [0.79–3.00]	.20	1.64 [0.82–3.30]	.16
CG+GG, n (%)	198 (40.9)	97 (39.9)		0.96 [0.71–1.31]	.82	0.97 [0.71–1.34]	.88
C-689T (n)	484	242					
CC, n (%)	374 (77.3)	193 (79.7)		reference		reference	
CT, n (%)	106 (21.9)	42 (17.4)	.04	0.76 [0.51–1.13]	.18	0.74 [0.49–1.13]	.16
TT, n (%)	4 (0.8)	7 (2.9)		3.34 [0.98–11.45]	.054	3.43 [0.96–12.27]	.058
CT+TT, n (%)	110 (22.7)	49 (20.3)		0.89 [0.61–1.30]	.54	0.87 [0.58–1.29]	.48
Pro12Ala (n)	486	245					
CC, n (%)	378 (77.8)	198 (80.8)		reference		reference	
CG, n (%)	104 (21.4)	40 (16.3)	.03	0.74 [0.49–1.10]	.14	0.72 [0.47–1.10]	.12
GG, n (%)	4 (0.8)	7 (2.9)		3.32 [0.97–11.39]	.056	3.41 [0.95–12.22]	.060
CG+GG, n (%)	108 (22.2)	47 (19.2)		0.85 [0.58–1.25]	.40	0.83 [0.55–1.24]	.36
C1431T (n)	482	241					
CC, n (%)	383 (79.5)	189 (78.4)		reference		reference	
CT, n (%)	97 (20.1)	46 (19.1)	.04	0.94 [0.64–1.38]	.76	0.89 [0.60–1.33]	.58
TT, n (%)	2 (0.4)	6 (2.5)		5.93 [1.19–29.45]	.029	5.10 [0.99–26.37]	.050
CT+TT, n (%)	99 (20.5)	52 (21.6)		1.01 [0.69–1.46]	.98	0.95 [0.64–1.40]	.78

(b)

	ADVANCE Men						
				Model 1		Model 2	
	Controls	Cases	*P**	OR [95% CI]	*P*	OR [95% CI]	*P*
C-681G (n)	420	694					
CC, n (%)	238 (56.7)	395 (56.9)		reference		reference	
CG, n (%)	159 (37.9)	264 (38.1)	.95	1.00 [0.78–1.29]	.99	1.03 [0.77–1.37]	.85
GG, n (%)	23 (5.4)	35 (5.0)		0.92 [0.53–1.59]	.76	0.85 [0.46–1.60]	.62
CG+GG, n (%)	182 (43.3)	299 (43.1)		0.99 [0.77–1.26]	.93	1.00 [0.76–1.32]	.99
C-689T (n)	423	687					
CC, n (%)	326 (77.1)	522 (76.0)		reference		reference	
CT, n (%)	93 (22.0)	154 (22.4)	.68	1.03 [0.77–1.39]	.82	1.00 [0.72–1.38]	.98
TT, n (%)	4 (0.9)	11 (1.6)		1.72 [0.54–5.44]	.35	1.87 [0.48–7.33]	.37
CT+TT, n (%)	97 (22.9)	165 (24.0)		1.06 [0.80–1.41]	.68	0.99 [0.78–1.26]	.94
Pro12Ala (n)	426	693					
CC, n (%)	330 (77.5)	528 (76.2)		reference		reference	
CG, n (%)	92 (21.6)	154 (22.2)	.62	1.05 [0.78–1.40]	.76	1.03 [0.74–1.42]	.88
GG, n (%)	4 (0.9)	11 (1.6)		1.72 [0.54–5.44]	.36	1.90 [0.49–7.41]	.36
CG+GG, n (%)	96 (22.5)	165 (23.8)		1.07 [0.81–1.43]	.62	1.06 [0.77–1.45]	.74
C1431T (n)	426	687					
CC, n (%)	325 (76.3)	530 (77.2)		reference		reference	
CT, n (%)	98 (23.0)	147 (21.4)	.50	0.92 [0.69–1.23]	.57	0.87 [0.63–1.21]	.41
TT, n (%)	3 (0.7)	10 (1.4)		2.01 [0.55–7.36]	.29	1.76 [0.43–7.16]	.43
CT+TT, n (%)	101 (23.7)	157 (22.8)		0.95 [0.71–1.25]	.73	0.90 [0.65–1.24]	.52

(c)

	ADVANCE Women						
				Model 1		Model 2	
	Controls	Cases	P^*	OR [95% CI]	P	OR [95% CI]	P
C-681G (n)	359	365					
CC, n (%)	189 (52.7)	203 (55.6)		reference		reference	
CG, n (%)	148 (41.2)	144 (39.5)	.64	0.91 [0.67–1.23]	.52	0.97 [0.68–1.38]	.86
GG, n (%)	22 (6.1)	18 (4.9)		0.76 [0.40–1.47]	.42	0.64 [0.30–1.4]	.26
CG+GG, n (%)	170 (47.3)	162 (44.4)		0.89 [0.66–1.19]	.42	0.92 [0.65–1.29]	.62
C-689T (n)	360	365					
CC, n (%)	274 (76.1)	285 (78.1)		reference		reference	
CT, n (%)	81 (22.5)	79 (21.6)	.26	0.94 [0.66–1.33]	.72	1.07 [0.71–1.6]	.74
TT, n (%)	5 (1.4)	1 (0.3)		0.19 [0.02–1.66]	.13	0.17 [0.02–1.54]	.12
CT+TT, n (%)	86 (23.9)	80 (21.9)		0.89 [0.63–1.26]	.53	1.00 [0.67–1.49]	.99
Pro12Ala (n)	362	366					
CC, n (%)	275 (76.0)	288 (78.7)		reference		reference	
CG, n (%)	82 (22.6)	77 (21.0)	.22	0.90 [0.63–1.28]	.54	1.02 [0.68–1.53]	.93
GG, n (%)	5 (1.4)	1 (0.3)		0.19 [0.02–1.65]	.13	0.168 [0.02–1.51]	.11
CG+GG, n (%)	87 (24.0)	78 (21.3)		0.86 [0.60–1.21]	.38	0.99 [0.67–1.49]	.80
C1431T (n)	366	367					
CC, n (%)	278 (76.0)	281 (76.6)		reference		reference	
CT, n (%)	81 (22.1)	83 (22.6)	.50	1.01 [0.72–1.44]	.94	1.08 [0.72–1.63]	.70
TT, n (%)	7 (1.9)	3 (0.8)		0.42 [0.11–1.66]	.21	0.30 [0.07–1.32]	.11
CT+TT, n (%)	88 (24.0)	86 (23.4)		0.97 [0.69–1.36]	.85	1.00 [0.67–1.49]	.96

*P value for a global test of significance. Model 1: crude OR. Model 2: OR adjusted for age, BMI, educational level, smoking status, physical activity, alcohol intake, history of diabetes, history of hypertension, and history of dyslipidemia.

test which was computed from the estimated haplotype frequency log-likelihoods for the case and control groups separately.

2.4.2. Meta-Analysis. In addition to the analysis of PRIME and ADVANCE, we identified all published prospective studies that assessed the relationship between the *PPARG* Pro12Ala polymorphism and CHD and conducted a meta-analysis of the findings. Inclusion criteria for events were a coronary event. Inclusion criteria for exposure variable were the *PPARG* Pro12Ala polymorphism. Searches were conducted in electronic databases (MEDLINE and EMBASE) from 1970 to June 2009. References from the extracted papers, reviews, and previous meta-analysis were also consulted to complete the data bank. The electronic search include both truncated free-text and MeSH. Used terms were "PPARG" or "peroxisome proliferator-activated receptor gamma" and "polymorphism" and "cardiovascular disease" or "CAD" or "CHD" or "coronary" or "MI" or "myocardial" or "ischemic heart disease." No attempt was made to contact authors of nonpublished work or to find paper in other languages than English. When the data were not available in the appropriate format, the corresponding author of the paper was contacted to obtain the data. However, two studies were excluded because the data were not available in the appropriate format, that is, the number of subjects for the three genotype groups in cases and controls [22, 23]. Finally, 11 studies were selected for the meta-analysis. Therefore, the final data set, including our 3 samples, consisted of 14 independent studies comprising 6898 cases and 11 287 controls (Table 4).

For this meta-analysis, we combined data of all studies using Review Manager software release 5.0 (http://www.cc-ims.net/revman/) [24]. We estimated the overall effect by a Mantel–Haentzel fixed odds ratio.

3. Results

As expected, major cardiovascular risk factors such as smoking, history of hypertension, history of dyslipidemia, history of diabetes, and systolic and diastolic blood pressure levels differed between cases and controls in both PRIME and ADVANCE studies (Table 1).

The genotype distributions of the 4 *PPARG* SNPs respected the Hardy-Weinberg equilibrium in control subjects. In the control group, the frequencies of the PPARG -681G, -689T, Ala12, and 1431T alleles were similar in PRIME and ADVANCE. There were marginal differences in the genotype distribution of the C-689T ($P = .04$), Pro12Ala ($P = .03$), and C1431T ($P = .04$) SNPs between cases

Table 3: Estimate of *PPARG* haplotype frequencies in cases and controls.

C-681G	C-689T	Pro12Ala	C1431T	Controls	Cases	*P*
PRIME Men						
1	1	1	1	0.72	0.72	
2	1	1	1	0.11	0.11	
2	2	2	2	0.08	0.09	.53
2	2	2	1	0.04	0.03	
1	1	1	2	0.03	0.03	
2	1	1	2	0.01	0.02	
ADVANCE Men						
1	1	1	1	0.74	0.73	
2	1	1	1	0.11	0.10	
2	2	2	2	0.09	0.08	.38
2	2	2	1	0.03	0.04	
1	1	1	2	0.02	0.02	
2	1	1	2	0.02	0.01	
ADVANCE Women						
1	1	1	1	0.71	0.72	
2	1	1	1	0.13	0.13	
2	2	2	2	0.08	0.08	.70
2	2	2	1	0.04	0.03	
1	1	1	2	0.03	0.03	
2	1	1	2	0.01	0.01	

Only haplotypes with a frequency >1% are displayed. 1 and 2 represent the frequent and minor alleles, respectively. *P* value is for the global effect (5 df).

and controls in PRIME (Table 2(a)), mainly due to a higher frequency of homozygote subjects for the minor allele in cases compared with controls.

The odds ratios (ORs) [95% CI] of CHD were calculated for heterozygotes, for homozygotes, and for carriers of the minor allele (dominant model), using both a crude model and an adjusted model (Table 2(a)). In PRIME, homozygote individuals for the minor allele of the *PPARG* C-689T, Pro12Ala, and C1431T SNPs tended to have a higher risk of CHD than homozygote individuals for the frequent allele (OR [95% CI] = 3.34 [0.98–11.45], P = .054, OR = 3.32 [0.97–11.39], P = .056 and OR = 5.93 [1.19–29.45], P = .029, resp.). After adjustment for age, educational level, smoking status, physical activity, alcohol intake, history of diabetes, history of hypertension, and history of dyslipidemia, the ORs were only slightly altered (OR = 3.43 [0.96–12.27], P = .058, 3.41 [0.95–12.22], P = .060 and 5.10 [0.99–26.37], P = .050 for the C-689T, Pro12Ala and C1431T SNPs, resp.). There was no significant association in ADVANCE, neither in men nor in women (Table 2(b) and (c)). Further analyses stratifying on overweight (<25 kg/m^2 versus ≥25 kg/m^2) or obesity (<30 kg/m^2 versus ≥30 kg/m^2) or adding a cross-product term into the model yielded no significant interaction (all P values for interaction term >0.1) in both studies (data not shown).

In control subjects from PRIME and ADVANCE, there were very few significant differences in baseline characteristics between carriers and noncarriers of the *PPARG*-681G, -689T, Ala12, and 1431T alleles (see Tables 1, 2, 3, 4, and 5 in Supplementary Material available online at doi:10.155/2009/543746). Systolic blood pressure was significantly lower (P < .05) in carriers of the *PPARG*-681G, -689T and Ala12 alleles in PRIME (Supplementary Table 1). There was no statistically significant difference in mean BMI, LDL-cholesterol, or HDL-cholesterol levels between carriers and noncarriers of the minor allele of any SNP investigated. There was also no evidence for any statistically significant difference in the prevalence of reported hypertension, dyslipidemia, or diabetes between carriers and noncarriers of the minor allele of these SNPs.

The Pro12Ala and the C-681G, C-689T, and C1431T SNPs were in partial positive linkage disequilibrium (D' range = +0.6 − +1.0, r^2 range = 0.18–0.98). The C-689T and Pro12Ala SNPs were in perfect linkage disequilibrium (D' = +1.0, r^2 = 0.98). The distribution of the 4 SNP -containing haplotypes was estimated in cases and controls (Table 3). Six haplotypes covered 98% of the possible haplotypes. The frequency of the haplotype composed of the 4 most frequent alleles was >70% in both cases and controls. There was no evidence for any statistically significant difference in haplotype distribution between cases and controls, neither in PRIME nor in ADVANCE (P > .38). Haplotype analyses were also conducted after stratification on overweight or obesity. In these analyses, there was no evidence of significant difference of haplotype distributions between cases and controls in overweight or lean, obese, or nonobese subjects (data not shown).

Pooled estimate of CHD odds ratios were calculated from published studies for the *PPARG* Ala12Pro SNP including 6898 cases and 11 287 controls. Funnel plot analysis revealed no evidence of publication bias. There was no significant association with CHD under the dominant genetic model (OR = 1.00 [0.93–1.08], P = .95) (Table 4(a)). There was significant heterogeneity in the meta-analysis (Chi^2 = 22.10, df = 13, P = .05) due to the study of Li et al., which was the only study to show a significant association between the Ala12 allele and a higher CHD risk. When removing this study from the meta-analysis (heterogeneity Chi^2 = 17.37, df = 12, P = .14), the odds ratio of CHD was 0.99 [0.92–1.07], P = .82. The ORs of CHD were similar in men (OR = 0.99, P = .91) and women (OR = 0.94, P = .52).

Using the recessive genetic model, there was a borderline association between the Ala12Ala genotype and CHD risk (OR = 1.29 [0.99–1.67], P = .06) (Table 4(b)). When stratifying on gender, Ala12Ala men had a significant higher risk of CHD (OR = 1.73 [1.20–2.48], P = .003) than men carrying the Pro12 allele (Table 4(c)). No such association could be detected in women (OR = 0.62 [0.31–1.24], P = .17) (data not shown).

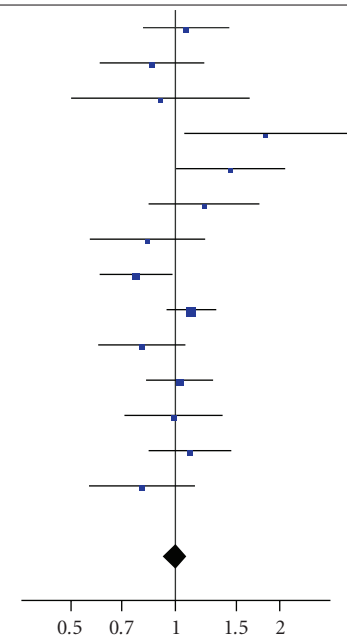
4. Discussion

In the present study, we did not find any consistent association between the *PPARG* C-681G, C-689T, Pro12Ala,

Table 4

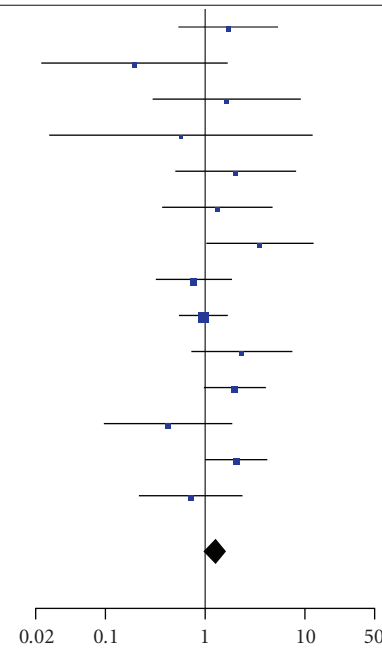
(a) Meta-analysis of CHD risk for the *PPARG* Pro12Ala polymorphism under the dominant model.

	Cases		Controls			Odds ratio	Odds ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
ADVANCE, Men	165	693	96	426	7.1%	1.07 [0.81, 1.43]	
ADVANCE, Women	78	366	87	362	5.4%	0.86 [0.60, 1.21]	
Bluher et al., Men + Women	27	201	24	164	1.8%	0.91 [0.50, 1.64]	
Li et al., Men + Women	23	218	38	626	1.4%	1.83 [1.06, 3.14]	
Pischon et al., Men	63	250	95	502	3.7%	1.44 [1.00, 2.07]	
Pischon et al., Women	58	245	99	485	4.0%	1.21 [0.84, 1.75]	
PRIME, Men	47	245	108	486	4.6%	0.83 [0.57, 1.22]	
Ridker et al., Men	98	523	482	2092	12.3%	0.77 [0.60, 0.98]	
Ruiz et al., Men + Women	365	1805	335	1805	20.9%	1.11 [0.94, 1.31]	
Tobin et al., Men + Women	113	547	124	505	8.0%	0.80 [0.60, 1.07]	
Vogel et al., Men	197	786	216	880	12.0%	1.03 [0.82, 1.28]	
Vogel et al., Women	64	245	208	789	5.7%	0.99 [0.71, 1.37]	
Vos et al., Men	126	563	134	646	7.6%	1.10 [0.84, 1.45]	
Zafarmand et al., Women	44	211	376	1519	5.7%	0.80 [0.56, 1.14]	
Total (95% CI)		6898		11287	100.0%	1.00 [0.93, 1.08]	
Total events	1468		2422				
Heterogeneity: $Chi^2 = 22.10$, df = 13 ($P = .05$); $I^2 = 41\%$							
Test for overall effect: $Z = 0.06$ ($P = .95$)							



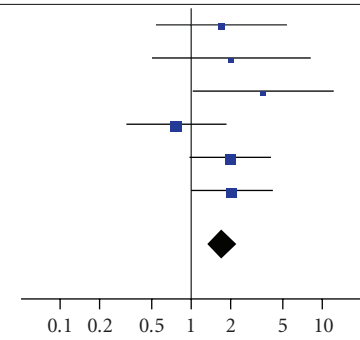
(b) Meta-analysis of CHD risk for the *PPARG* Pro12Ala polymorphism under the recessive model.

	Cases		Controls			Odds ratio	Odds ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
ADVANCE, Men	11	693	4	426	4.9%	1.70 [0.54, 5.38]	
ADVANCE, Women	1	366	5	362	5.0%	0.20 [0.02, 1.68]	
Bluher et al., Men + Women	4	201	2	164	2.2%	1.64 [0.30, 9.09]	
Li et al., Men + Women	0	218	2	626	1.3%	0.57 [0.03, 11.95]	
Pischon et al., Men	4	250	4	502	2.6%	2.02 [0.50, 8.16]	
Pischon et al., Women	4	245	6	485	4.0%	1.33 [0.37, 4.74]	
PRIME, Men	7	245	4	486	2.6%	3.54 [1.03, 12.23]	
Ridker et al., Men	6	523	31	2092	12.3%	0.77 [0.32, 1.86]	
Ruiz et al., Men + Women	24	1805	25	1805	24.8%	0.96 [0.55, 1.69]	
Tobin et al., Men + Women	10	547	4	505	4.1%	2.33 [0.73, 7.48]	
Vogel et al., Men	21	786	12	880	11.1%	1.99 [0.97, 4.06]	
Vogel et al., Women	2	245	15	789	7.1%	0.42 [0.10, 1.87]	
Vos et al., Men	21	563	12	646	10.8%	2.05 [1.00, 4.20]	
Zafarmand et al., Women	3	211	30	1519	7.2%	0.72 [0.22, 2.37]	
Total (95% CI)		6898		11287	100.0%	1.29 [0.99, 1.67]	
Total events	118		156				
Heterogeneity: $Chi^2 = 15.93$, df = 13 ($P = .25$); $I^2 = 18\%$							
Test for overall effect: $Z = 1.90$ ($P = .06$)							



(c) Meta-analysis of CHD risk for the *PPARG* Pro12Ala polymorphism under the recessive model in men.

	Cases		Controls			Odds ratio	Odds ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
ADVANCE, Men	11	693	4	426	11.0%	1.70 [0.54, 5.38]	
Pischon et al., Men	4	250	4	502	5.9%	2.02 [0.50, 8.16]	
PRIME, Men	7	245	4	486	5.9%	3.54 [1.03, 12.23]	
Ridker et al., Men	6	523	31	2092	27.8%	0.77 [0.32, 1.86]	
Vogel et al., Men	21	786	12	880	25.0%	1.99 [0.97, 4.06]	
Vos et al., Men	21	563	12	646	24.4%	2.05 [1.00, 4.20]	
Total (95% CI)		3060		5032	100.0%	1.73 [1.20, 2.48]	
Total events	70		67				
Heterogeneity: $Chi^2 = 4.93$, df = 5 ($P = .42$); $I^2 = 0\%$							
Test for overall effect: $Z = 2.96$ ($P = .003$)							



or C1431T SNPs or related haplotypes and CHD risk, neither in men nor in women, in the PRIME and ADVANCE studies. Furthermore, analyses stratified on overweight or obesity revealed no evidence that these *PPARG* variants influence CHD risk in overweight or obese men. These data do not support the hypothesis that *PPARG* C-681G, C-689T, Pro12Ala, and C1431T polymorphisms are major risk factors for CHD but does not rule out the possibility of an association of small magnitude, especially in homozygote Ala12Ala men.

PPARG is a ligand-activated transcription factor playing an important role in adipocyte differentiation, glucose homeostasis, and several vascular processes. In vitro studies have shown that the *PPARG* Ala12 allele decreases PPARG activation of reporter genes (4) and the -681G and -689T alleles are associated with lower PPARG promoter activity [4, 6, 7] suggesting that carriers of these alleles might have a lower PPARG-mediated activation of target genes than noncarriers.

Earlier clinical and epidemiological studies have assessed the relationship between variability at the *PPARG* gene loci and CHD risk. The results of these investigations have been inconsistent with some studies showing a reduced risk of coronary heart disease [9, 22], other studies an increased risk [25–29] and some others reporting no association [10, 23, 30–33]. In contrast, three studies showed less intima-media carotid thickening in carriers of the *PPARG* Ala12 allele suggesting a possible relation between this variant and sub-clinical atherosclerosis [34–36].

We found no consistent association for none of the 4 *PPARG* SNPs (C-681G, C-689T, Pro12Ala, and C1431T) in PRIME and ADVANCE. Only in PRIME, we observed that men homozygotes for the minor allele of the C-689T, Pro12Ala, and C1431T SNPs had a higher risk of CHD than homozygotes for the frequent allele. This result was further confirmed in the meta-analysis including 3060 cases and 5032 controls that showed a higher risk of CHD in Ala12Ala men. Recent genome whole association studies failed to identify associations with *PPARG* SNPs [37–42]. Firstly, this might be explained by the gender-specific effect and the restrictive recessive genetic model of the *PPARG* association. Secondly, the 4 SNPs that were investigated in the present study may not be the culprit ones, but rather be in linkage disequilibrium with a causal mutation, resulting in a dilution of a possible association.

PPARG SNPs may affect CHD risk through their association with cardiovascular risk factors. In the present study, we found no evidence for significant differences in major cardiovascular risk factors for example, body mass index, lipid levels, or history of diabetes, between carriers and noncarriers of the minor alleles. The only exception was a borderline association with systolic blood pressure level which was lower in *PPARG*-681G, -689T, and Ala12 carriers than in noncarriers in the PRIME study and could confer some protection against CHD. These findings are in agreement with other studies which showed lower levels of blood pressure or risk of hypertension in carriers of the minor allele of the *PPARG* Pro12Ala SNP [43–45]. However, these associations are inconsistent across studies as some also reported higher levels [46, 47] or not significantly different levels [48, 49] of blood pressure between carriers and noncarriers of the *PPARG* Ala12 allele. In this sample of apparently healthy men, the prevalence of hypertension was low (~18%) and the impact of the *PPARG*-681G, -689T, and Ala12 alleles on blood pressure was clinically limited.

An earlier study has reported a significant higher risk of MI and fatal CHD in overweight but not in normal weight subjects [10] suggesting that the impact of *PPARG* genotype may differ according to body fat mass. In the present study, which has a similar number of cases and controls, we found no evidence for such a relationship, suggesting that obesity is not a determinant of the association between *PPARG* genetic variability and CHD.

5. Conclusion

In the present study, there was no major consistent association between the *PPARG* C-681G, C-689T, Pro12Ala, and C1431T genotypes or related haplotypes and CHD risk. When combining our data with previous cohort studies, focusing on the *PPARG* Pro12Ala SNP, we found a significant association between the homozygote Ala12Ala genotype and a higher risk of CHD in men. Further studies are needed to definitely conclude.

The PRIME Study Group

The Strasbourg MONICA Project, Department of Epidemiology and Public Health, Faculty of Medicine, Strasbourg, France (D. Arveiler, B. Haas).

The Toulouse MONICA Project, INSERM U558, Department of Epidemiology, Paul Sabatier-Toulouse Purpan University, Toulouse, France (J. Ferrières, JB. Ruidavets).

The Lille MONICA Project, INSERM U744, Pasteur Institute, Lille, France (P. Amouyel, M. Montaye).

The Department of Epidemiology and Public Health, Queen's University, Belfast, Northern Ireland (A. Evans, J. Yarnell, F. Kee).

The Department of Atherosclerosis, INSERM U545, Lille, France (G. Luc, JM. Bard).

The Laboratory of Haematology, INSERM U626, La Timone Hospital, Marseilles, France (I. Juhan-Vague, Pierre Morange).

The Laboratory of Endocrinology, INSERM U563, Toulouse, France (B. Perret).

The Vitamin Research Unit, The University of Bern, Bern, Switzerland (F. Gey).

The Trace Element Laboratory, Department of Medicine, Queen's University Belfast, Northern Ireland (Jayne Woodside, Ian Young).

The DNA Bank, INSERM U525, Paris, France (F. Cambien).

The Coordinating Center, INSERM U780 (ex-U258), Paris-Villejuif, France (P. Ducimetière, A. Bingham).

Acknowledgments

The authors thank the following organizations which allowed the recruitment of the PRIME subjects: the Health Screening Centers organized by the Social Security of Lille (Institut Pasteur), Strasbourg, Toulouse, and Tourcoing; Occupational Medicine Services of Haute-Garonne, the Urban Community of Strasbourg, the Association interentreprises des services médicaux du travail de Lille et environs; the Comité pour le développement de la médecine du travail; and the Mutuelle Générale des PTT du Bas-Rhin. They also thank the participants in the ADVANCE study. The PRIME study was supported by unrestricted grants from Merck Sharp & Dohme-Chibret (France) and from the Department of Health and Social Service (Northern Ireland) and by The Fondation de France. The ADVANCE study was supported by a grant from the Donald W. Reynolds Foundation.

References

[1] M. Lehrke and M. A. Lazar, "The many faces of PPARγ," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[2] J. N. Feige, L. Gelman, L. Michalik, B. Desvergne, and W. Wahli, "From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions," *Progress in Lipid Research*, vol. 45, no. 2, pp. 120–159, 2006.

[3] R. Kostadinova, W. Wahli, and L. Michalik, "PPARs in diseases: control mechanisms of inflammation," *Current Medicinal Chemistry*, vol. 12, no. 25, pp. 2995–3009, 2005.

[4] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in PPARγ2associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[5] A. Meirhaeghe, L. Fajas, N. Helbecque, et al., "A genetic polymorphism of the peroxisome proliferator-activated receptor γ gene influences plasma leptin levels in obese humans," *Human Molecular Genetics*, vol. 7, no. 3, pp. 435–440, 1998.

[6] A. Meirhaeghe, L. Fajas, F. Gouilleux, et al., "A functional polymorphism in a STAT5B site of the human PPARγ3 gene promoter affects height and lipid metabolism in a French population," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 2, pp. 289–294, 2003.

[7] A. Meirhaeghe, M. W. T. Tanck, L. Fajas, et al., "Study of a new PPARγ2 promoter polymorphism and haplotype analysis in a French population," *Molecular Genetics and Metabolism*, vol. 85, no. 2, pp. 140–148, 2005.

[8] A. Meirhaeghe, D. Cottel, P. Amouyel, and J. Dallongeville, "Association between peroxisome proliferator-activated receptor γ haplotypes and the metabolic syndrome in French men and women," *Diabetes*, vol. 54, no. 10, pp. 3043–3048, 2005.

[9] P. M. Ridker, N. R. Cook, S. Cheng, et al., "Alanine for proline substitution in the peroxisome proliferator-activated receptor gamma-2 (PPARG2) gene and the risk of incident myocardial infarction," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 5, pp. 859–863, 2003.

[10] T. Pischon, J. K. Pai, J. E. Manson, et al., "Peroxisome proliferator-activated receptor-γ2P12A polymorphism and risk of coronary heart disease in US men and women," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 8, pp. 1654–1658, 2005.

[11] J. W. G. Yarnell, "The PRIME study: classical risk factors do not explain the severalfold differences in risk of coronary heart disease between France and Northern Ireland," *QJM*, vol. 91, no. 10, pp. 667–676, 1998.

[12] P. Ducimetiere, J.-B Ruidavets, M. Montaye, B. Haas, and J. Yarnell, "Five-year incidence of angina pectoris and other forms of coronary heart disease in healthy men aged 50–59 in France and Northern Ireland: the prospective epidemiological study of myocardial infarction (PRIME) study," *International Journal of Epidemiology*, vol. 30, no. 5, pp. 1057–1062, 2001.

[13] G. H. Hughes, G. Cutter, R. Donahue, et al., "Recruitment in the coronary artery disease risk development in young adults (cardia) study," *Controlled Clinical Trials*, vol. 8, no. 4, supplement 1, pp. 68S–73S, 1987.

[14] A. S. Go, C. Iribarren, M. Chandra, et al., "Statin and β-blocker therapy and the initial presentation of coronary heart disease," *Annals of Internal Medicine*, vol. 144, no. 4, pp. 229–238, 2006.

[15] C. Iribarren, A. S. Go, G. Husson, et al., "Metabolic syndrome and early-onset coronary artery disease. is the whole greater than its parts?" *Journal of the American College of Cardiology*, vol. 48, no. 9, pp. 1800–1807, 2006.

[16] M. A. Hlatky, E. Ashley, T. Quertermous, et al., "Matrix metalloproteinase circulating levels, genetic polymorphisms, and susceptibility to acute myocardial infarction among patients with coronary artery disease," *American Heart Journal*, vol. 154, no. 6, pp. 1043–1051, 2007.

[17] T. L. Assimes, J. W. Knowles, A. Basu, et al., "Susceptibility locus for clinical and subclinical coronary artery disease at

chromosome 9p21 in the multi-ethnic ADVANCE study," *Human Molecular Genetics*, vol. 17, no. 15, pp. 2320–2328, 2008.

[18] M. Slatkin and L. Excoffier, "Testing for linkage disequilibrium in genotypic data using the Expectation-Maximization algorithm," *Heredity*, vol. 76, no. 4, pp. 377–383, 1996.

[19] R. C. Lewontin, "The interaction of selection and linkage. I. General considerations; heterotic models," *Genetics*, vol. 49, no. 1, pp. 49–67, 1964.

[20] D. A. Tregouet, S. Barbaux, S. Escolano, et al., "Specific haplotypes of the P-selectin gene are associated with myocardial infarction," *Human Molecular Genetics*, vol. 11, no. 17, pp. 2015–2023, 2002.

[21] D. A. Tregouet, S. Escolano, L. Tiret, A. Mallet, and J. L. Golmard, "A new algorithm for haplotype-based association analysis: the stochastic-EM algorithm," *Annals of Human Genetics*, vol. 68, no. 2, pp. 165–177, 2004.

[22] A. S. Doney, B. Fischer, G. Leese, A. D. Morris, and C. N. A. Palmer, "Cardiovascular risk in type 2 diabetes is associated with variation at the PPARG locus: a go-DARTS study," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 12, pp. 2403–2407, 2004.

[23] L. Gallicchio, B. Kalesan, H. Y. Huang, P. Strickland, S. C. Hoffman, and K. J. Helzlsouer, "Genetic polymorphisms of peroxisome proliferator-activated receptors and the risk of cardiovascular morbidity and mortality in a community-based cohort in washington county, Maryland," *PPAR Research*, vol. 2008, Article ID 276581, 9 pages, 2008.

[24] Review Manager (RevMan) [Computer program], Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008.

[25] C. C. Szeto, K.-M. Chow, P. Y.-K. Poon, B. C.-H. Kwan, and P. K.-T. Li, "Peroxisome proliferator-activated receptor-gamma gene polymorphism and risk of cardiovascular disease in patients with diabetic nephropathy," *American Journal of Nephrology*, vol. 28, no. 5, pp. 715–722, 2008.

[26] E. A. Ruiz-Narváez, P. Kraft, and H. Campos, "Ala12 variant of the peroxisome proliferator-activated receptor-γ gene (PPARG) is associated with higher polyunsaturated fat in adipose tissue and attenuates the protective effect of polyunsaturated fat intake on the risk of myocardial infarction," *American Journal of Clinical Nutrition*, vol. 86, no. 4, pp. 1238–1242, 2007.

[27] L. Li, L.-X. Cheng, R. Nsenga, M.-A. He, and T.-C. Wu, "Association between Pro 12Ala polymorphism of peroxisome proliferator-activated receptor-gamma 2 and myocardial infarction in the Chinese Han population," *Clinical Cardiology*, vol. 29, no. 7, pp. 300–304, 2006.

[28] M. D. Tobin, P. S. Braund, P. R. Burton, et al., "Genotypes and haplotypes predisposing to myocardial infarction: a multilocus case-control study," *European Heart Journal*, vol. 25, no. 6, pp. 459–467, 2004.

[29] U. Vogel, S. Segel, C. Dethlefsen, et al., "PPARgamma Pro12Ala polymorphism and risk of acute coronary syndrome in a prospective study of Danes," *BMC Medical Genetics*, vol. 10, p. 52, 2009.

[30] L. Gallicchio, H. Chang, D. K. Christo, et al., "Single nucleotide polymorphisms in inflammation-related genes and mortality in a community-based cohort in Washington County, Maryland," *American Journal of Epidemiology*, vol. 167, no. 7, pp. 807–813, 2008.

[31] M. H. Zafarmand, Y. T. van der Schouw, D. E. Grobbee, P. W. de Leeuw, and M. L. Bots, "Peroxisome proliferator-activated receptor gamma-2 P12A polymorphism and risk of acute myocardial infarction, coronary heart disease and ischemic stroke: a case-cohort study and meta-analyses," *Vascular Health and Risk Management*, vol. 4, no. 2, pp. 427–436, 2008.

[32] E. J. Rhee, C. H. Kwon, W. Y. Lee, et al., "No association of Pro12Ala polymorphism of PPAR-γ gene with coronary artery disease in Korean subjects," *Circulation Journal*, vol. 71, no. 3, pp. 338–342, 2007.

[33] M. Bluher, T. Klemm, T. Gerike, H. Krankenberg, G. Schuler, and R. Paschke, "Lack of association between peroxisome proliferator-activated receptor-γ-2 gene variants and the occurrence of coronary heart disease in patients with diabetes mellitus," *European Journal of Endocrinology*, vol. 146, no. 4, pp. 545–551, 2002.

[34] E. Iwata, I. Yamamoto, T. Motomura, et al., "The association of Pro12Ala polymorphism in PPARγ2 with lower carotid artery IMT in Japanese," *Diabetes Research and Clinical Practice*, vol. 62, no. 1, pp. 55–59, 2003.

[35] T. Temelkova-Kurktschiev, M. Hanefeld, G. Chinetti, et al., "Ala12Ala genotype of the peroxisome proliferator-activated receptor γ2 protects against atherosclerosis," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 9, pp. 4238–4242, 2004.

[36] K. Z. Al-Shali, A. A. House, A. J. G. Hanley, et al., "Genetic variation in PPARG encoding peroxisome proliferator-activated receptor γ associated with carotid atherosclerosis," *Stroke*, vol. 35, no. 9, pp. 2036–2040, 2004.

[37] R. McPherson, A. Pertsemlidis, N. Kavaslar, et al., "A common allele on chromosome 9 associated with coronary heart disease," *Science*, vol. 316, no. 5830, pp. 1488–1491, 2007.

[38] A. Helgadottir, G. Thorleifsson, A. Manolescu, et al., "A common variant on chromosome 9p21 affects the risk of myocardial infarction," *Science*, vol. 316, no. 5830, pp. 1491–1493, 2007.

[39] N. J. Samani, J. Erdmann, A. S. Hall, et al., "Genomewide association analysis of coronary artery disease," *The New England Journal of Medicine*, vol. 357, no. 5, pp. 443–453, 2007.

[40] P. R. Burton, D. G. Clayton, L. R. Cardon, et al., "Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls," *Nature*, vol. 447, no. 7145, pp. 661–678, 2007.

[41] J. Erdmann, A. Grosshennig, P. S. Braund, et al., "New susceptibility locus for coronary artery disease on chromosome 3q22.3," *Nature Genetics*, vol. 41, no. 3, pp. 280–282, 2009.

[42] D. A. Tregouet, I. R. Konig, J. Erdmann, et al., "Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease," *Nature Genetics*, vol. 41, no. 3, pp. 283–285, 2009.

[43] M. Horiki, H. Ikegami, T. Fujisawa, et al., "Association of Pro12Ala polymorphism of PPARgamma gene with insulin resistance and related diseases," *Diabetes Research and Clinical Practice*, vol. 66, supplement 1, pp. S63–S67, 2004.

[44] F. J. Rodríguez-Esparragón, J. C. Rodríguez-Pérez, A. Macías-Reyes, and F. Alamo-Santana, "Peroxisome proliferator-activated receptor-gamma2-Pro12Ala and endothelial nitric oxide synthase-4a/b gene polymorphisms are associated with essential hypertension," *Journal of Hypertension*, vol. 21, no. 9, pp. 1649–1655, 2003.

[45] C. J. Östgren, U. Lindblad, O. Melander, A. Melander, L. Groop, and L. Råstam, "Peroxisome proliferator-activated receptor-γ Pro12Ala polymorphism and the association with blood pressure in type 2 diabetes: skaraborg hypertension and diabetes project," *Journal of Hypertension*, vol. 21, no. 9, pp. 1657–1662, 2003.

[46] S. J. Hasstedt, Q.-F. Ren, K. Teng, and S. C. Elbein, "Effect of the peroxisome proliferator-activated receptor-γ2 Pro12Ala variant on obesity, glucose homeostasis, and blood pressure in members of familial type 2 diabetic kindreds," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 2, pp. 536–541, 2001.

[47] J. A. Douglas, M. R. Erdos, R. M. Watanabe, et al., "The peroxisome poliferator—activated receptor-γ2 Pro 12Ala variant: association with type 2 diabetes and trait differences," *Diabetes*, vol. 50, no. 4, pp. 886–890, 2001.

[48] Y. Mori, H. Kim-Motoyama, T. Katakura, et al., "Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor γ2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men," *Biochemical and Biophysical Research Communications*, vol. 251, no. 1, pp. 195–198, 1998.

[49] I. Gouni-Berthold, E. Giannakidou, D. Müller-Wieland, et al., "Peroxisome proliferator-activated receptor-γ2 Pro12Ala and endothelial nitric oxide synthase-4a/b gene polymorphisms are not associated with hypertension in diabetes mellitus type 2," *Journal of Hypertension*, vol. 23, no. 2, pp. 301–308, 2005.

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 786781, 5 pages
doi:10.1155/2009/786781

Research Article

Genetic Variability of RXRB, PPARA, and PPARG in Wegener's Granulomatosis

Stefan Wieczorek,[1] Silvia Knaup,[1] Wolfgang L. Gross,[2] and Jörg T. Epplen[1]

[1] *Human Genetics Department, Ruhr University, 44801 Bochum, Germany*
[2] *Department of Rheumatology, University Hospital Lübeck and Klinikum Bad Bramstedt, 24576 Bad Bramstedt, Germany*

Correspondence should be addressed to Stefan Wieczorek, stefan.wieczorek@rub.de

Received 29 August 2008; Revised 4 December 2008; Accepted 5 January 2009

Recommended by Mostafa Badr

A major genomic region involved in Wegener's granulomatosis includes the gene for retinoid receptor beta (*RXRB*) which forms heterodimers with peroxisome proliferator-activated receptors (PPARs). It is unclear whether this association directly arises from the *RXRB* allele(s) or via a linked variation. In order to reveal any hitherto unknown and potentially disease-relevant variation of the *RXRB* gene, we have genotyped four tagging SNPs of this genomic region and have directly sequenced selected WG patients and controls representing disease-associated haplotypes. Additionally, we have genotyped 2 SNPs each in the genes for PPARα and PPARγ (*PPARA* and *PPARG*). Hence, we confirmed the strong association of the *RXRB* locus with WG but could not reveal any novel variation in *RXRB*. None of the *PPARA* and *PPARG* SNPs showed association with WG. Moreover, no epistatic effect was seen between *RXRB* and *PPARA*/*PPARG* alleles. These results do not support an etiopathological role of PPAR in WG. Analyses of further genes functionally linked to RXRB may provide additional data useful to evaluate the *RXRB* association found in WG.

1. Introduction

Wegener's granulomatosis (WG) is a form of small vessel vasculitis belonging to the group antineutrophil cytoplasmic antibody- (ANCA-) associated vasculitides (AAV). WG usually presents with granulomatous lesions of the upper respiratory tract and often develops into generalized vasculitis with multiple organ involvement in later disease stages. In WG ANCA typically recognize proteinase 3, delineating this diagnostic entity from other AAV forms like Churg Strauss syndrome or microscopic polyangiitis, in which ANCA are usually directed against myeloperoxidase (reviewed in [1]). Current concepts of its etiopathology are incomplete, but WG is accounted to the large and heterogenous group of complex disorders arise from a mostly elusive interplay of environmental and genetic factors.

A major genomic locus for WG was identified on chromosome 6p21.3, including (among others) the genes for *HLA-DPB1, RXRB*, and *RING1* [2]. The linkage disequilibrium (LD) pattern of this genomic region is complex with larger blocks of strong LD alternating with regions of high recombination rates. There is evidence that the association of this locus with the disorder may arise from more than one variation, making 6p21.3 a quantitative trait locus for WG. While a strong association is demonstrable with the *HLA-DPB1**0104 allele, a (partly) independent association was detected for the defined region including potential WG candidate genes *RING1* and *RXRB* [3]. In addition certain alleles of *RXRB*, the gene encoding retinoid X receptor beta, are highly significantly associated with WG susceptibility [4].

Peroxisome proliferator-activated receptors (PPAR) are group of nuclear receptors mediating the effects of peroxisome proliferators on gene transcription. Binding of PPAR to regulatory DNA sequences requires the heterodimerization with RXR. PPAR mediated effects are complex and include modulation of inflammatory processes. PPARα is a ligand for leukotriene B4, and PPARα deficient mice show a prolonged response to inflammatory stimuli [5]. PPARα also appears to be involved in downregulation of the activity of cyclooxigenase 2 (COX2) and nuclear factor kappa-b (NFκB). Likewise, PPARγ exhibits anti-inflammatory properties. It is upregulated in activated macrophages and inhibits

the expression, for example, of the inducible nitric oxide synthase [6].

Genetic polymorphisms of PPAR have been investigated in numerous conditions, predominantly in metabolic disorders like type 2 diabetes or atherosclerosis [7–9]. There is evidence for accelerated atherosclerosis in WG patients [10], suggesting that both conditions may share some (genetic) risk factors.

Given the pleiotropic role of PPAR in the regulation of (vascular) inflammation, we have hypothesised that genetic predisposition to WG may arise from the interaction of certain genetic variations of RXRB and PPAR. We have, therefore, screened the *RXRB* gene for novel, potentially WG-specific variations. Moreover, we have genotyped two single nucleotide polymorphisms in each of the genes for PPARα and PPARγ in large panels of WG cases and healthy controls. Finally, we searched for epistatic effects of certain alleles of *RXRB* and *PPARA* or *PPARG*.

2. Patients and Methods

2.1. Subjects. All patients included in this study were diagnosed with WG according to the criteria of the *American College of Rheumatology* and the Chapel Hill consensus conference [11]. They were selected at the interdisciplinary vasculitis centre at the University of Luebeck/Rheumaklinik Bad Bramstedt. All patients were asked for their ancestry and reported German decent for at least two generations. Healthy German blood donors were used as controls and ancestry was evaluated equal to the patient group.

For the *RXRB* locus we genotyped the same patients (n = 282) and controls (n = 380) that were previously analyzed for the 6p21.3 locus [3]. In order to increase statistical power, the SNP in the PPAR genes were analyzed in expanded sample comprising 462 WG cases and 701 controls.

Ethical principles for medical research involving human subjects as defined in the *Declaration of Helsinki* have been followed. The study design was approved by the local ethics committee at the University of Luebeck, Germany (No. AZ 06-087).

2.2. Genotyping. Four types SNPs rs9277935, rs2072915, rs2744537, and rs1547387 (see Figure 1) were selected from the HapMap database to serve as tagSNPs for a region of 10 kb including the *RXRB* gene. With this selection all HapMap SNPs within this region are efficiently tagged ($r^2 > 0.9$). rs2072915 and rs2744537 are located within the 3′ untranslated region (UTR) of *RXRB,* while rs9277935 is located approximately 1 kb 3′ of the last *RXRB* exon. rs1547387 is located 5′ of *RXRB*, in exon 5 of the *SLC39A7* gene, in which it constitutes a silent SNP (Ser209Ser).

rs9277935, rs2072915, and rs2744537 were genotyped via PCR-RFLP techniques and a commercially available TaqMan genotyping assay (Applied Biosystems) was used for rs1547387.

For rs1800206 (Val162Leu) in *PPARA* also a commercially available TaqMan assay was used, while rs6008259 (3′ UTR of *PPARA*), rs1801282 (*PPARG*, Ala12Pro), and rs3856806 (*PPARG*, His449His) were genotyped using newly designed primers and probes (see Table 1).

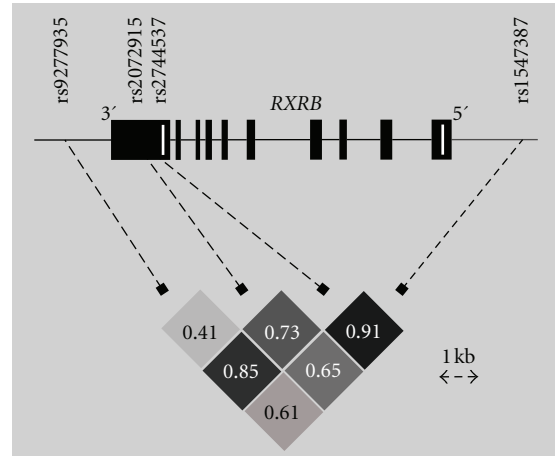


Figure 1: Location of the four tagging SNPs of the *RXRB* gene and LD structure. The LD is plotted as D' and intensity coded (darker shades corresponding to higher degree of LD). The *RXRB* gene is transcribed from centromer to telomer, that is, from right to left. *RXRB* exons are depicted as black boxes, and translation start and stop points are marked by white vertical lines.

2.3. Direct Sequencing of RXRB. The entire *RXRB* gene (all 10 exons and exon/intron boundaries) was directly sequenced in 5 WG patients typed homozygous for the associated *RXRB* haplotype (see below; primer sequences available on request). For comparison 5 healthy controls homozygous for the inversely associated (i.e., protective) haplotype were also sequenced.

2.4. Statistical Analysis. Genotypes were recorded in linkage format. Association for each single marker was tested by using chi square tests on contingency tables. A P value <.05 was considered significant. LD between each SNP of a respective locus, and haplotype block frequencies were calculated by using Haploview 4.1 [12].

Interaction between *RXRB* and *PPARA*/*PPARG* SNPs were calculated with GAIA (http://gump.qimr.edu.au/GAIA/gaia.html; [13]). This application uses a logistic regression model which tests for pairwise locus/locus interactions between genes. We applied an additive interaction model for each pair of SNPs testing the significance of the interaction model terms over and above any main effects.

3. Results and Discussion

The overall call rate for all 8 SNPs in patients and controls was 93.60%. None of the SNPs revealed significant deviation from Hardy-Weinberg equilibrium. Two of the *RXRB* SNPs (rs9277935 and rs2744537) showed highly significant association with WG (see Table 2). Accordingly, the GTTC haplotype (calculated from all four SNPs of this locus) was significantly more common in WG while the TTGC haplotype was overrepresented in controls (see Table 2).

Table 1: Oligonucleotides and restriction enzymes used for genotyping.

Gene	SNP	Forward (F) and reverse (R) primer (5′ > 3′)	Allele specific probes	Restriction enzyme	Fragment lengths
RXRB	rs9277935	F: TGCCCCTTGTAG-GTCTCCAC (1)	—	BtgI	T-Allel: 208 bp
	T/G	R: CCTCCCACTGTG-CCCTAA			G-Allel: 34 + 174 bp
	rs2072915	F: ACATCTCCACCA-GCCCCTTC	—	MboI	T-Allel: 325 bp
	T/A	R: GTCCTTCCCCCA-GCACAAAG			A-Allel: 224 + 101 bp
	rs2744537	F: TCTTCAAGCTCA-TTGGTGAC	—	BsaI	T-Allel: 451 bp
	T/G	R: CCCATTTCCACT-CTTCAGAT			G-Allel: 326 + 125 bp
	rs1547387	Information not provided by the manufacturer		—	—
	C/G				
PPARA	rs1800206	Information not provided by the manufacturer		—	—
	G/C				
	rs6008259	F: CCCCTGTGTCAA-CAAGATCCA	G: Fam-CTGTGTTGTCC-CCAGCGACCC	—	—
	G/A	R: CTTGAATGGCAC-AGGGTACATC	A: YY-CCTGTGTTGTCC-CCAACGACCC		
PPARG	rs1801282	F: TTATGGGTGAAA-CTCTGGGAGATT	G: Fam-TCCTATTGACG-CAGAAAGCGATTCC	—	—
	G/C	R: TTGTGATATGTT-TGCAGACAGTGTATC	C: YY-TCCTATTGACCC-AGAAAGCGATTCCTT		
	rs3856806	F: CCAGAAAATGAC-AGACCTCAGACA	T: Fam-TCACGGAACAT-GTGCAGCTACTGC	—	—
	T/C	R: GGAGCGGGTGAA-GACTCATG	C: YY-ATTGTCACGGAA-CACGTGCAGCTAC		

(1) A 17 mer nucleotide (GTAAAACGACGGCCAGT) was added 5′ to the forward primer increase fragment length differences between the two alleles, FAM: 6-carboxy-fluorescein; YY: Yakima Yellow.

Based on the SNP data we have then selected 5 WG patients homozygous for the GTTC haplotype for direct sequencing of the *RXRB* gene. For comparison 5 control subjects homozygous for the TTGC haplotype were also analyzed. Yet, no novel sequence variation was detected, a finding which is consistent with previous results from Szyld et al. [4], who had screened the *RXRB* gene in WG patients without consideration of the haplotype structure of this locus. Results from the initial tagging SNP genotyping were confirmed in the ten included individuals for the two exonic tagging SNPs (rs2744537 and rs2072915). Two other exonic SNPs (rs1152296 in exon 10/5′UTR and rs6531 in exon 7) were identified and perfectly segregated with the previously identified risk/nonrisk haplotypes.

Both associated *RXRB* SNPs (rs9277935 and rs2744537) are in strong LD with *HLA-DPB1* as well as with rs3117228 and rs213208 (see Figure 2) which had revealed the highest (and partly *HLA-DPB1* independent) association with WG in a previous study [3]. Moreover, according to the HapMap data rs2744537 is in strong LD with rs6531 (a synonymous SNP in exon 7 of *RXRB*) which was significantly associated with WG earlier [4]. As for the other associated SNPs in this genomic region, it is therefore hard to differentiate which of the SNPs (or even a yet unknown variation of this locus) constitutes the primary WG risk factor. A potential approach to overcome this problem is the analysis of factors related to the different proteins encoded in the 6p21.3 region. We have therefore investigated SNPs in the genes for PPARα and PPARγ which are functionally closely linked to RXRB.

The two SNPs in *PPARG* were in weak to moderate LD ($D' = 0.64$, $r^2 = 0.40$) while the two SNPs in *PPARA* were virtually unlinked ($D' = 0.47$, $r^2 = 0.06$). Therefore, no haplotype frequencies were calculated for these genes. Both SNPs in *PPARG* showed very similar allele frequencies in cases and controls not revealing any significant differences (see Table 2). The two SNPs in *PPARA* both revealed a

Table 2: Allele and haplotype frequencies.

Gene	SNP/haplotype	Allele	Frequency in controls	Frequency WG patients	*P* value	OR (95% CI)
RXRB	rs9277935	G	0.76	0.89	6.65×10^{-9}	2.55 (1.86–3.56)
		T	0.24	0.11		
	rs2072915	T	0.71	0.75	.157	1.20 (0.93–1.55)
		A	0.29	0.25		
	rs2744537	T	0.73	0.57	6.63×10^{-9}	0.49 (0.39–0.63)
		G	0.27	0.43		
	rs1547387	C	0.87	0.85	.34	0.85 (0.62–1.18)
		G	0.13	0.15		
*RXRB**	GTTC		0.24	0.40	1.15×10^{-9}	2.11 (1.66–2.69)
	TTGC		0.19	0.07	9.73×10^{-10}	0.32 (0.22–0.47)
	other		0.43	0.47	.157	1.18 (0.94–1.47)
PPARA	rs1800206	C	0.93	0.95	.076	1.40 (0.96–2.05)
		G	0.07	0.05		
	rs6008259	G	0.80	0.83	.069	0.81 (0.65–1.02)
		A	0.20	0.17		
PPARG	rs1801282	C	0.85	0.85	.74	0.96 (0.75–1.23)
		G	0.15	0.15		
	rs3856806	C	0.86	0.85	.70	0.95 (0.75–1.22)
		T	0.14	0.15		

OR: Odds ratio,
CI: confidence interval,
*haplotype frequencies calculated from all four *RXRB* SNPs.

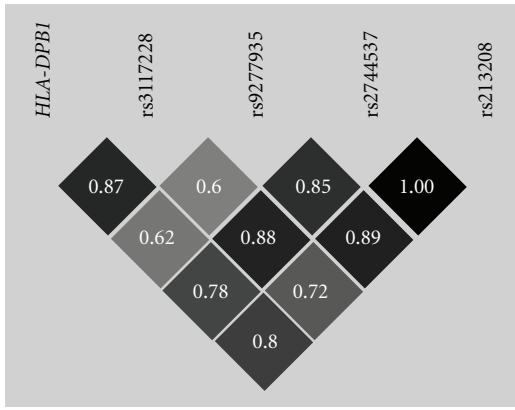


Figure 2: LD between WG associated variations of the 6p21.3 genomic region. *HLA-DPB1* is modelled as a biallelic marker with the associated *HLA-DPB1**0401 as one allele and all other alleles joint into the other allelic category. LD is plotted as *D*′ and intensity coded (darker shades corresponding to higher degree of LD). Both *RXRB* SNPs investigated in this study (rs9277935 and rs2744537) are in high LD with *HLA-DPB1* as well as with the markers analyzed by Heckmann et al. (2008): rs3117228 (3′ of *HLA-DPB1*) and rs213208 (intronic SNP in *RING1*, approx. 10 kb centromeric of *RXRB*).

decreased frequency of the minor allele but these differences did not reach significance level (see Table 2). No significant pairwise epistatic effect for any of the investigated SNPs, that is, neither between *PPARA* and *PPARG* nor between *PPARA* (or *PPARG*, resp.) and *RXRB* or *HLA-DPB1*. Taken together, we cannot provide evidence for a genetically based involvement of PPARα and PPARγ in the etiopathology of WG. Moreover, the strong WG association with the 6p21.3 locus is unlikely to be based on any coding variation of the *RXRB* gene. Future studies will therefore have to focus on regulatory elements of this area (e.g., *cis* acting elements or micro RNAs).

4. Conclusions

These results do not support a direct etiopathological role of RXRB and/or PPAR in WG. Analyses of further genes functionally linked to RXRB, for example, retinoic acid receptors or vitamin D receptors, may provide additional data useful to evaluate the *RXRB* association found in WG.

Acknowledgment

This work was supported by the Deutsche Forschungsgemeinschaft [KFO 170].

References

[1] E. Csernok, F. Moosig, and W. L. Gross, "Pathways to ANCA production: from differentiation of dendritic cells by proteinase 3 to B lymphocyte maturation in Wegener's

granuloma," *Clinical Reviews in Allergy & Immunology*, vol. 34, no. 3, pp. 300–306, 2008.

[2] P. Jagiello, M. Gencik, L. Arning, et al., "New genomic region for Wegener's granulomatosis as revealed by an extended association screen with 202 apoptosis-related genes," *Human Genetics*, vol. 114, no. 5, pp. 468–477, 2004.

[3] M. Heckmann, J. U. Holle, L. Arning, et al., "The Wegener's granulomatosis quantitative trait locus on chromosome 6p21.3 as characterised by tagSNP genotyping," *Annals of the Rheumatic Diseases*, vol. 67, no. 7, pp. 972–979, 2008.

[4] P. Szyld, P. Jagiello, E. Csernok, W. L. Gross, and J. T. Epplen, "On the Wegener granulomatosis associated region on chromosome 6p21.3," *BMC Medical Genetics*, vol. 7, article 21, pp. 1–11, 2006.

[5] P. R. Devchand, H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli, "The PPARα-leukotriene B_4 pathway to inflammation control," *Nature*, vol. 384, no. 6604, pp. 39–43, 1996.

[6] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[7] K. A. Volcik, J. A. Nettleton, C. M. Ballantyne, and E. Boerwinkle, "Peroxisome proliferator-activated receptor α genetic variation interacts with n-6 and long-chain n-3 fatty acid intake to affect total cholesterol and LDL-cholesterol concentrations in the Atherosclerosis Risk in Communities Study," *The American Journal of Clinical Nutrition*, vol. 87, no. 6, pp. 1926–1931, 2008.

[8] L. Dongxia, H. Qi, L. Lisong, and G. Jincheng, "Association of peroxisome proliferator-activated receptorγ gene Pro12Ala and C161T polymorphisms with metabolic syndrome," *Circulation Journal*, vol. 72, no. 4, pp. 551–557, 2008.

[9] M.-J. Shin, A. M. Kanaya, and R. M. Krauss, "Polymorphisms in the peroxisome proliferator activated receptor α gene are associated with levels of apolipoprotein CIII and triglyceride in African-Americans but not Caucasians," *Atherosclerosis*, vol. 198, no. 2, pp. 313–319, 2008.

[10] K. de Leeuw, J.-S. Sanders, C. Stegeman, A. Smit, C. G. Kallenberg, and M. Bijl, "Accelerated atherosclerosis in patients with Wegener's granulomatosis," *Annals of the Rheumatic Diseases*, vol. 64, no. 5, pp. 753–759, 2005.

[11] J. C. Jennette, R. J. Falk, K. Andrassy, et al., "Nomenclature of systemic vasculitides: proposal of an international consensus conference," *Arthritis & Rheumatism*, vol. 37, no. 2, pp. 187–192, 1994.

[12] J. C. Barrett, B. Fry, J. Maller, and M. J. Daly, "Haploview: analysis and visualization of LD and haplotype maps," *Bioinformatics*, vol. 21, no. 2, pp. 263–265, 2005.

[13] S. Macgregor and I. A. Khan, "GAIA: an easy-to-use web-based application for interaction analysis of case-control data," *BMC Medical Genetics*, vol. 7, article 34, pp. 1–6, 2006.

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 874126, 5 pages
doi:10.1155/2009/874126

Research Article

Impact of the Pro12Ala Polymorphism of the PPAR-Gamma 2 Gene on Metabolic and Clinical Characteristics in the Palestinian Type 2 Diabetic Patients

S. Ereqat,[1,2] A. Nasereddin,[2] K. Azmi,[2] Z. Abdeen,[2] and R. Amin[1]

[1] *Department of Biochemistry and Molecular Biology, Medical School, Al-Quds University, East Jerusalem, Abu-Deis, P.O. Box 19356, Palestine*
[2] *Al-Quds Nutrition and Health Research Institute, Al-Quds University, East Jerusalem, P.O. Box 20760, Palestine*

Correspondence should be addressed to A. Nasereddin, abedn@ekmd.huji.ac.il

Received 14 June 2009; Revised 6 August 2009; Accepted 25 August 2009

Recommended by Mostafa Badr

Peroxisome proliferators activated receptor-gamma2 (PPARγ2) represents the transcriptional master regulator of adipocyte differentiation and therefore has been suggested as a candidate gene for obesity, insulin resistance, and dyslipidemia. The objective of the study was to investigate for the first time the potential association of the most common variant Pro12Ala (p.P12A) substitution of the *PPARγ2* gene with body mass index (BMI), blood pressure, fasting plasma glucose, plasma total cholesterol, LDL and HDL cholesterol, and plasma triglyceride in a sample of 202 (138 females and 64 male) type 2 diabetic Palestinians. Genotyping of the *PPARγ2* p.P12A polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The A12 allele was associated with lower fasting plasma glucose ($P = .03$) but had no influence on blood pressure, BMI, or other metabolic parameters. In obese patients, the p.P12A substitution was associated with elevated total plasma cholesterol levels ($P = .02$) and a tendency toward increased LDL cholesterol level ($P = .06$). In conclusion, the p.P12A variant of the *PPARγ2* may influence cardiovascular risk through effects on lipid metabolism in obese T2D Palestinian patients.

1. Introduction

Type 2 diabetes mellitus (T2DM) showed to be a cocktail of genetic discovery as described by Freeman and Cox [1]. T2DM cases would have inborn with a diversity of different genetic factors that together with environmental factors combine as the primary cause and contributed to the disease.

Studies enlightening the epidemiology of diabetes mellitus among Palestinians are scarce. A Palestinian study conducted at 2001 [2] showed DM prevalence in 12% of urban Palestinian population including 492 men and women 30–65 years old. According to the WHO global estimate, and the epidemic nature of diabetes; prevalence of diabetes is expected to increase in Palestine and numbers should be revised to have more realistic estimation, which enables health providers to be aware of the disease magnitude, so that more effective health strategies can be adopted.

Preoxisome proliferator-activated receptor gamma (PPARγ) is a nuclear hormone receptor which plays a critical role in regulating adipocyte differentiation and the transcription of genes that are important for lipogenesis. In macrophages, the activated PPARγ promotes the cellular efflux of phospholipids and cholesterol in the form of high density lipoprotein (HDL) [3, 4]. PPAR$\gamma 2$ is a key modulator of adipogenesis and insulin signaling [5]. Cytosine to guanine single nucleotide polymorphism (rs1805192) in PPAR$\gamma 2$ gene (NM_015869) resulting in a proline to alanine substitution at codon 12, which has been found to modulate the transcriptional activity of the gene [6], and associated with altered insulin sensitivity [7]. Although most studies have shown a statistically significant T2DM risk reduction given by Ala variant [8–11], some others have not [12–15], suggesting variability in the contribution of this variant to the risk of T2DM. In a previous study of 333 Palestinian subjects ($n = 219$ T2DM patients and $n = 114$

Table 1: Anthropometrical and biochemical parameters of the study groups. Data are in means ± SD. BMI =Body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC: total cholesterol; TG: Triglyceride; FPG: fasting plasma glucose; NS: not significant; D: Diet; I: insulin; IO: Insulin and oral hypoglycemic agent; O: oral hypoglycemic agent.

Parameters	Obese subjects $n = 121$	Nonobese subjects $n = 81$	P
Sex (M/F)	31/90	33/48	
Age (years)	58.6 + 10.1	59.9 + 10.1	NS
Age at diagnosis	49.8 + 10.1	49.6 + 10.5	NS
BMI (kg/m^2)	35.4 + 4.9	25.8 + 2.5	<.001
SBP (mmHg)	126.1 + 18.8	130.1 + 19.0	NS
DBP (mmHg)	78.6 + 9.3	79.6 + 14.8	NS
FPG (mg/dL)	208.4 + 84.0	211.3 + 80.3	NS
TC (mg/dL)	201.5 + 52.6	189.6 + 44.3	NS
TG (mg/dL)	181.4 + 78.9	167.5 + 83.5	NS
HDL (mg/dL)	43.6 + 16.0	41.3 + 13.4	NS
LDL (mg/dL)	120.1 + 48.5	109.6 + 44.9	NS
Therapy(n) D/I/IO/O	4/22/16/79	2/22/5/52	NS

normoglycemic controls), we were unable to demonstrate a significant association between the p.P12A variant and the T2DM among Palestinians (0.055 versus 0.048, OR = 0.87, $P = .9$) (unpublished data). The aim of this study was to investigate for the first time the relationship between p.P12A polymorphism of PPAR$\gamma 2$ gene and blood pressure, BMI and other related metabolic parameters in type 2 diabetic Palestinians.

2. Subjects and Methods

After an institutional review board approval and a completion of an informed consent, two hundred and two (202) diabetic type 2 patient volunteers (121 obese subjects (BMI $\geq$ 30 kg/m^2) and 81 nonobese subjects (BMI $<$ 30 kg/m^2) were randomly recruited on the basis of routine visits to the Amari Refugee camp outpatient UNRWA clinic in the Ramallah District, 14 km north Jerusalem. The study protocol and the consent form were approved by the institutional Human Research Ethics Committee at Al-Quds University. The volunteers were unrelated, over 40 years old and diagnosed with diabetes according to WHO criteria or currently being treated with medication for diabetes. Subjects with previous diagnosis of type 1 diabetes were excluded from the study. Complete medical records including family history for diabetes, resting blood pressure, and anthropometric parameters: weight, height, and BMI (weight in kilograms divided by the square of height in meters) were obtained.

For laboratory analysis, 5 mL of blood were obtained after overnight fast. Blood samples were collected in EDTA-tubes and immediately centrifuged at room temperature. Plasma was aliquoted for glucose, cholesterol, HDL-cholesterol, and triglyceride determination. Plasma glucose was determined by a glucose oxidase method. Plasma total cholesterol, triglyceride and HDL cholesterol were determined by enzymatic methods using commercial kits (Human, Wiesbaden, Germany). Plasma LDL cholesterol was calculated by the Friedewald formula [16]. All the biochemical measures were obtained in milligram/deciliter (mg/dL), to convert plasma glucose, triglyceride, and cholesterol values into millimolar concentration (mmol/L). Multiply the given values in (mg/dL) units by 0.0555, 0.0113, and 0.0259, respectively.

Genomic DNA was extracted from peripheral blood according to standard procedures (Epicenter, Madison, Wis, USA). The p.P12A polymorphism was detected by PCR-RFLP analysis as previously described by Johansen et al. [17] with the following modifications: The PCR reactions were carried out using 100 ng of purified genomic DNA samples, with 1 μM of each primer (forward/reverse) and PCR-Ready Supreme mix (Syntezza Bioscience, Jerusalem) in a final volume of 25 μl.

3. Statistical Analysis

Statistical differences between mean values of the continuous variables of subjects with and without the polymorphism were evaluated by student's t test and confirmed by a nonparametric analysis using Mann-Whitney test. Chi-square analysis was performed to test the allelic frequency differences between obese and nonobese subjects. Hardy-Weinberg equilibrium was computed to the expected genotype distribution. Analysis was performed using SPSS program version 15. P value less than .05 was considered significant.

4. Results

The biochemical and anthropometric results for the 121 obese subjects and 81 nonobese subjects are shown in Table 1. No significant differences in biochemical parameters

were noted between the two groups. The genotype analysis in nonobese subjects revealed that 73 individuals were homozygote for the wild P12P allele, and 8 individuals were heterozygote P12A. Among the obese group, 106 individuals were homozygote for the wild P12P allele, 14 individuals were heterozygote P12A and only one patient was homozygote A12A for the rare allele. Because there was only one homozygote patient for the rare allele, this individual was combined with the heterozygote group and compared with the homozygote wild group in all of the statistical analyses. No significant statistical correlation was observed between medication for diabetes and either genotype or gender ($P > .05$) in both groups. The overall frequency of the A12 allele in T2DM patients was 0.059 and it was in Hardy-Weinberg equilibrium. Since PPAR$\gamma 2$ is an important regulator of adipogenesis, it was relevant to verify if the p.P12A variant was associated with obesity. The frequency of the A12 allele did not differ significantly between obese and nonobese diabetic subjects (0.066 versus 0.049, $P = 0.6$). No significant differences in the genotypic distribution were found between the two groups as shown in Table 2. The presence of A12 variant was significantly associated with lower plasma glucose (174 ± 45.9 vresus 214 ± 84.9 mg/dL, $P = .03$) for the whole group (Table 3). However, there were no significant statistical differences between the PPAR$\gamma 2$ genotype and gender, age, BMI, plasma triglyceride, plasma total cholesterol, HDL and LDL cholesterol, as well as systolic and diastolic blood pressure.

Among obese subjects, p.P12A carriers showed significantly higher level of plasma total cholesterol (232.2 ± 41.8 versus 197.1 ± 52.7 mg/dL, $P = .02$) and tended with a borderline significant to have lower plasma glucose than those with P12P genotype ($P = .05$). A tendency toward increased levels of LDL cholesterol among P12A genotype were also observed but not significant ($P = .06$) (Table 3). Both subgroups showed no association of P12A genotype and triglyceride, and HDL cholesterol.

Table 2: PPAR$\gamma 2$ genotype frequencies in obese and nonobese Type 2 diabetic patients. The chi-square test was performed for association.

	Genotype (n)		
	P12P	P12A	A12 frequency
Obese	106	15	0.066
Nonobese	73	8	0.049
All subjects	179	23	0.059

5. Discussion

In this study, the frequency of the A12 allele of the PPAR$\gamma 2$ gene in diabetic subjects was similar to that reported for other Arabic populations such as Qatari and Tunisian ones [13, 14], but was slightly higher than in East Asian (Malaysians, Chinese, and Koreans) populations [15, 18] and much lower than that reported in Caucasian subjects [19]. This might be explained by the genetic background shared between our studied group and the other Arabic origin subgroups [13, 14] compared with other ethnic groups [15, 18, 19]. More comprehensive genotyping studies are needed to clarify this point. Of at most interest is to recruit and genotype large representative Palestinian T2DM patients to get conclusive results for comparison with the Caucasian group.

In agreement with early published studies, we could not demonstrate an effect of the p.P12A variant on several traits that are associated with insulin resistant syndrome, such the BMI, blood pressure, and plasma triglyceride levels [15, 20].

On the other hand, our study showed the diabetic subjects with the P12A genotype had lower fasting plasma glucose levels than those with the P12P genotype. This effect had a borderline significance in the obese group. Thamer et al. [21] indicated that the mechanism by which the A12 allele improves insulin sensitivity might involve enhanced suppression of lipid oxidation permitting more efficient glucose disposal, however, this effect should be clarified in future studies with detailed laboratory tests such as glycated hemoglobin and insulin sensitivity measures by the gold standard OGTT and/or the hyperinsulinemic euglycemic clamp [22].

Of the variables tested, the P12A genotype had the greatest effect on plasma total cholesterol with an elevated level among diabetic obese than nonobese subjects consistent with early published studies in Caucasian and Japanese populations [8, 19]. Plasma LDL cholesterol exhibited a similar trend, but did not achieve significance. Since both obese and nonobese groups had similar lipid profile (Table 1). This effect is not simply reflected by obesity, it might be revealed due to variable interactions of the Ala allele with other genetic and environmental factors. In the Finnish study, the effect of P12A genotype on T2DM risk was modified by physical activity [23]. Combining all these results together, it may suggest an intrinsic reduction in the PPAR$\gamma 2$ activity; however, it was shown that the A12 substitution in the PPAR$\gamma 2$ gene associated with reduced lipoprotein lipase activity [24] which results in impaired clearance of LDL, VLDL resulting in elevated total plasma cholesterol, LDL, and VLDL levels. In contrast, other studies did not find impact of p.P12A of PPAR$\gamma 2$ on lipid profile in diabetic and normoglycemic subjects [25]. We believe that ethnic differences, BMI, gender, physical activities, study design, and sample size all could be considered as reasons for discrepancies in association studies.

In conclusion, this study is considered to be the first to focus on molecular type-2 diabetes on Palestinians which will help in draw a baseline for further diabetes research studies in Palestine. Our findings showed no significant impact of the p.P12A PPAR$\gamma 2$ polymorphism on BMI, blood pressure and triglyceride. However, this variant in obese T2DM patients may influence cardiovascular risk through effects on lipid metabolism. The role of other polymorphisms particularly—if found—in a location near to the p.P12A is still to be defined in our population.

Table 3: Biochemical and anthropometrical parameters for subjects based on PPARγ2 genotype. The chi-square test was performed for association.

	All subjects			Obese subjects		
	P12P	P12A	*P*	P12P	P12A	*P*
Age (years)	58.8 ± 10.0	61.4 ± 10.5	NS	58.3 + 10.4	61.3 + 7.6	NS
BMI (kg/m^2)	31.5 ± 6.2	31.5 ± 6.7	NS	35.4 + 4.9	35.1 + 4.9	NS
FPG (mg/dL)	214.1 ± 84.9	174.3 ± 45.9	.03	214.1 ± 86.8	168.5 ± 45.5	.05
TC (mg/dL)	192.5 ± 51.0	211.1 ± 50.2	NS	197.1 ± 52.7	232.2 ± 41.8	.02
TG (mg/dL)	176.1 ± 82.2	173.6 ± 71.3	NS	181.9 ± 79.0	177.0 ± 80.6	NS
HDL (mg/dL)	42.5 ± 15.3	43.6 ± 13.5	NS	43.9 ± 16.2	41.8 ± 15.1	NS
LDL (mg/dL)	114.8 ± 47.7	124.2 ± 43.7	NS	117.0 ± 49.3	141.9 ± 37.4	.06
SBP (mmHg)	127.5 ± 18.5	129.4 ± 22.3	NS	125.7 + 18.6	128.4 + 20.6	NS
DBP (mmHg)	78.7 ± 11.6	81.0 ± 11.0	NS	78.0 + 8.9	82.8 + 11.1	NS

Acknowledgments

The authors would like to thank Dr. Umaya Khamash, Chief Field Health Program, and the UNRWA outpatient clinic TEAM at Amari Refugee camp in Ramallah for their support throughout the sample collections. The authors thank Dr. Adel Shalata for his critical review of the manuscript.

References

[1] H. Freeman and R. D. Cox, "Type-2 diabetes: a cocktail of genetic discovery," *Human Molecular Genetics*, vol. 15, supplement 2, pp. R202–R209, 2006.

[2] H. F. Abdul-Rahim, A. Husseini, R. Giacaman, J. Jervell, and E. Bjertness, "Diabetes mellitus in an urban Palestinian population: prevalence and associated factors," *Eastern Mediterranean Health Journal*, vol. 7, no. 1-2, pp. 67–78, 2001.

[3] W. He, "*PPAR*γ2$^{\text{Pro12Ala}}$ polymorphism and human health," *PPAR Research*, vol. 2009, Article ID 849538, 15 pages, 2009.

[4] M.-C. Cho, K. Lee, S.-G. Paik, and D.-Y. Yoon, "Peroxisome proliferators-activated receptor (PPAR) modulators and metabolic disorders," *PPAR Research*, vol. 2008, Article ID 679137, 14 pages, 2008.

[5] M. Stumvoll and H. Häring, "The peroxisome proliferator-activated receptor-γ2 Pro12Ala polymorphism," *Diabetes*, vol. 51, no. 8, pp. 2341–2347, 2002.

[6] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in *PPAR*γ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[7] J. Ek, G. Andersen, S. A. Urhammer, et al., "Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 (*PPAR*-γ2) gene in relation to insulin sensitivity among glucose tolerant Caucasians," *Diabetologia*, vol. 44, no. 9, pp. 1170–1176, 2001.

[8] H. Mori, H. Ikegami, Y. Kawaguchi, et al., "The Pro12 → Ala substitution in PPAR-γ 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*, vol. 50, no. 4, pp. 891–894, 2001.

[9] K. Hara, T. Okada, K. Tobe, et al., "The Pro12Ala polymorphism in *PPAR*γ2 may confer resistance to type 2 diabetes," *Biochemical and Biophysical Research Communications*, vol. 271, no. 1, pp. 212–216, 2000.

[10] E. Zeggini, J. R. C. Parkinson, S. Halford, et al., "Examining the relationships between the Pro12Ala variant in *PPARG* and type 2 diabetes-related traits in UK samples," *Diabetic Medicine*, vol. 22, no. 12, pp. 1696–1700, 2005.

[11] M. Ghoussaini, D. Meyre, S. Lobbens, et al., "Implication of the Pro12Ala polymorphism of the *PPAR-gamma*2 gene in type 2 diabetes and obesity in the French population," *BMC Medical Genetics*, vol. 6, article 11, 2005.

[12] S. K. Hansen, E.-M. D. Nielsen, J. Ek, et al., "Analysis of separate and combined effects of common variation in *KCNJ11* and *PPARG* on risk of type 2 diabetes," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 6, pp. 3629–3637, 2005.

[13] R. Badii, A. Bener, M. Zirie, et al., "Lack of association between the Pro$_{12}$Ala polymorphism of the *PPAR*-γ2 gene and type 2 diabetes mellitus in the Qatari consanguineous population," *Acta Diabetologica*, vol. 45, no. 1, pp. 15–21, 2008.

[14] K. Z. Bouassida, L. Chouchane, K. Jellouli, et al., "The peroxisome proliterator activated receptorφ_2 (*PPAR*φ_2) Pro12Ala variant: lack of association with type 2 diabetes in obese and non obese Tunisian patients," *Diabetes & Metabolism*, vol. 31, no. 2, pp. 119–123, 2005.

[15] E. Y. Oh, K. M. Min, J. H. Chung, et al., "Significance of Pro^{12}Ala mutation in peroxisome proliferator-activated receptor-γ2 in Korean diabetic and obese subjects," *The Journal of Clinical Endocrinology & Metabolism*, vol. 85, no. 5, pp. 1801–1804, 2000.

[16] W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clinical Chemistry*, vol. 18, no. 6, pp. 499–502, 1972.

[17] A. Johansen, D. P. Jensen, R. Bergholdt, et al., "*IRS1*, *KCNJ11*, *PPAR*γ2 and *HNF-1*α: do amino acid polymorphisms in these candidate genes support a shared aetiology between type 1 and type 2 diabetes?" *Diabetes, Obesity and Metabolism*, vol. 8, no. 1, pp. 75–82, 2006.

[18] E. S. Tai, D. Corella, M. Deurenberg-Yap, et al., "Differential effects of the C1431T and Pro12Ala *PPAR*γ gene variants on plasma lipids and diabetes risk in an Asian population," *Journal of Lipid Research*, vol. 45, no. 4, pp. 674–685, 2004.

[19] B. Zietz, N. Barth, D. Spiegel, G. Schmitz, J. Schölmerich, and A. Schäffler, "Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-γ2 (*PPAR*γ2) is associated with higher levels of total cholesterol and LDL-cholesterol in male Caucasian type 2 diabetes patients," *Experimental and Clinical Endocrinology and Diabetes*, vol. 110, no. 2, pp. 60–66, 2002.

[20] J. Ringel, S. Engeli, A. Distler, and A. M. Sharma, "Pro12Ala missense mutation of the peroxisome proliferator activated receptor γ and diabetes mellitus," *Biochemical and Biophysical Research Communications*, vol. 254, no. 2, pp. 450–453, 1999.

[21] C. Thamer, M. Haap, A. Volk, et al., "Evidence for greater oxidative substrate flexibility in male carriers of the Pro 12 Ala polymorphism in PPARγ2," *Hormone and Metabolic Research*, vol. 34, no. 3, pp. 132–136, 2002.

[22] R. A. DeFronzo, J. D. Tobin, and R. Andres, "Glucose clamp technique: a method for quantifying insulin secretion and resistance," *The American Journal of Physiology*, vol. 237, no. 3, pp. E214–E223, 1979.

[23] T. O. Kilpeläinen, T. A. Lakka, D. E. Laaksonen, et al., "SNPs in *PPARG* associate with type 2 diabetes and interact with physical activity," *Medicine and Science in Sports & Exercise*, vol. 40, no. 1, pp. 25–33, 2008.

[24] J. Schneider, J. Kreuzer, A. Hamann, P. P. Nawroth, and K. A. Dugi, "The Proline 12 substitution in the peroxisome proliferator-activated receptor-γ2 gene is associated with lower lipoprotein lipase activity in vivo," *Diabetes*, vol. 51, no. 3, pp. 867–870, 2002.

[25] F. P. Mancini, O. Vaccaro, L. Sabatino, et al., "Pro12Ala substitution in the peroxisome proliferator-activated receptor-γ2 is not associated with type 2 diabetes," *Diabetes*, vol. 48, no. 7, pp. 1466–1468, 1999.

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 849538, 15 pages
doi:10.1155/2009/849538

Review Article

PPAR$\gamma 2^{\mathbf{Pro12Ala}}$ Polymorphism and Human Health

Weimin He

Alkek Institute of Biosciences and Technology, Texas A&M University System Health Science Center, 2121 W. Holcombe Blvd., Houston, TX 77030, USA

Correspondence should be addressed to Weimin He, whe@ibt.tamhsc.edu

Received 31 December 2008; Accepted 17 February 2009

Recommended by Marie-Claude Vohl

The nuclear hormone receptor peroxisome proliferator activated receptor gamma (PPARγ) is an important transcription factor regulating adipocyte differentiation, lipid and glucose homeostasis, and insulin sensitivity. Numerous genetic mutations of PPARγ have been identified and these mutations positively or negatively regulate insulin sensitivity. Among these, a relatively common polymorphism of PPARγ, Pro12Ala of PPARγ2, the isoform expressed only in adipose tissue has been shown to be associated with lower body mass index, enhanced insulin sensitivity, and resistance to the risk of type 2 diabetes in human subjects carrying this mutation. Subsequent studies in different ethnic populations, however, have revealed conflicting results, suggesting a complex interaction between the PPARγ2 Pro12Ala polymorphism and environmental factors such as the ratio of dietary unsaturated fatty acids to saturated fatty acids and/or between the PPARγ2 Pro12Ala polymorphism and genetic factors such as polymorphic mutations in other genes. In addition, this polymorphic mutation in PPARγ2 is associated with other aspects of human diseases, including cancers, polycystic ovary syndrome, Alzheimer disease and aging. This review will highlight findings from recent studies.

1. Introduction

Peroxisome proliferator activator receptor gamma (PPARγ) is a member of the nuclear hormone receptor superfamily that transcriptionally regulates genes controlling a variety of biological functions including cell growth, differentiation, and metabolism in response to lipophilic hormones, dietary fatty acids, and their metabolites [1]. Unlike some steroid hormone receptors such as the estrogen receptor, that are bound by heat shock proteins and sequestered in the cytoplasm, PPARγ is constitutively localized in the nucleus [2], heterodimerizes with the retinoid X receptor (RXR) [3], and binds to corepressors [4]. Ligand binding results in a conformational change in the receptor, triggering dissociation of corepressor complex and recruitment of coactivator proteins, leading to activation of gene expression [4].

Human PPARγ gene is located in chromosome 3 and spans a genomic segment of >150 kb. It consists of 9 exons (A1, A2, B, and 1–6), from which the two distinct isoforms of PPARγ mRNA and protein, PPARγ1 and PPARγ2, are derived through the use of separate promoters and 5′ exons. PPARγ1 mRNA specie is comprised of exons A1, A2, and 1–6, and is translated from P1 promoter while PPARγ2 mRNA is a combination of exons B and 1–6 and is translated from P2 promoter. The two proteins differ by the presence of extra 28 amino acids at the NH_2-terminus of PPARγ2 [5, 6]. PPARγ is abundantly expressed in adipose tissue, colon and macrophages while its expression is much lower in skeletal muscle, heart and other tissues [7, 8]. PPARγ1 is ubiquitously expressed whereas PPARγ2 expression is restricted to adipose tissue [9] (Figure 1).

PPARγ plays many functional roles in different organs and tissues (Figure 2). In vivo and in vitro studies demonstrate its critical role in regulating adipocyte differentiation and promoting lipid accumulation in adipose tissue [10–13]. It is also important for maintaining the viability and normal function of differentiated adipocytes [14–16]. In macrophages, PPARγ may enhance foam cell formation and atherogenesis upon increased uptake of oxidized low-density lipoprotein (oxLDL) [17, 18] or increases liver X receptor (LXR)-ATP-binding cassette A1 (ABCA1)-dependent cholesterol efflux upon pharmacological activation by its agonist TZDs [19, 20]. PPARγ in macrophages has also been shown

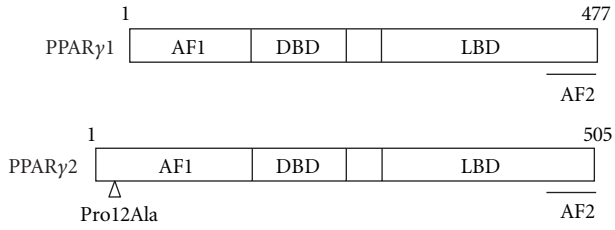


Figure 1: Domain structure of human PPARγ. AF1, activation function 1; DBD, DNA binding domain; LBD, ligand binding domain; AF2, activation function 2.

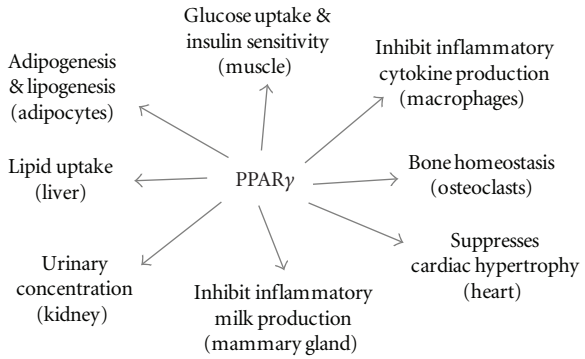


Figure 2: Pleiotropic functions of PPARγ in different organs/tissues.

to be involved in suppression of inflammatory cytokine production [21, 22] and improvement of insulin sensitivity [23, 24]. PPARγ in Skeletal muscle critically regulates normal glucose metabolism in muscle and lipid homeostasis in fat and the liver [25, 26] while PPARγ in the liver is implicated in controlling systemic glucose and lipid metabolism [27, 28]. PPARγ also plays roles in regulating bone homeostasis [29], heart hypertrophy [30, 31], high fat diet-induced hypertension [32], and urine concentration in the kidney (Cao et al., unpublished data).

PPARγ is also intimately implicated in regulation of glucose and lipid homeostasis and insulin sensitivity [33–35]. Not surprisingly, PPARγ has been identified as the target for thiazolidinediones (TZDs) [36], a class of synthetic compounds that improve insulin sensitivity in a variety of insulin resistant animal models and diabetic patients [33–35]. This role of PPARγ in affecting insulin action is consistent with many human genetic studies with various single amino acid mutations, including Pro12Ala, Pro115 Gln, Cys114Arg, Cyc131Tyr, Cyc162Trp, Val290Met, Pro388Leu, Arg425Cyc, His477His, and Pro467Leu that are scattered in activation function domain 1 (AF1), DNA binding domain (DBD), or ligand binding domain (LBD) of the receptor [37–45]. These mutations result in either gain-of-function or loss-of-function of the receptor; human subjects bearing these mutations show decreased or increased lipid accumulation in adipose tissue, enhanced insulin sensitivity or insulin resistance, dyslipidemia, diabetes, and hypertension [46–50]. Among these, Pro12Ala mutation in PPARγ2

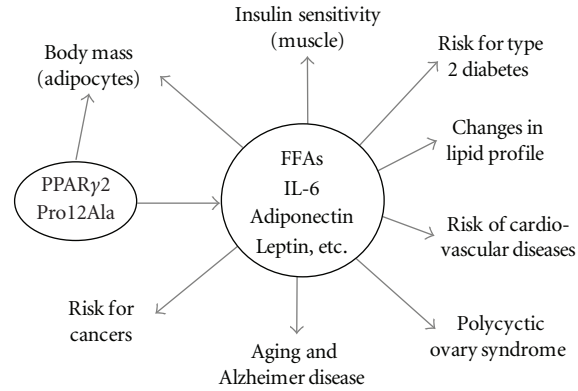


Figure 3: Effects of PPARγ2^{Pro12Ala} polymorphism on various aspects of human health. FFAs, free fatty acids, IL-6, interleukin 6.

(PPARγ2^{Pro12Ala}) is the most common. This mutation was first identified by Schuldiner's group in 1997 [37], with different ethnic populations showing various allelic frequencies. Caucasians have the highest frequency (12%), followed by Mexican Americans (10%), West Samoans (8%), African Americans (3%) while Chinese have the lowest (1%) [37]. In the last 10 years, extensive studies have been undertaken to assess the effects of this polymorphism on many aspects of human physiology (Figure 3). This review will summarize the effect of this mutation on human health revealed in these studies.

2. Effect of PPARγ2^{Pro12Ala} on Adiposity

Soon after the identification of Pro12Ala mutation, an independent study demonstrates that Ala12 variant is associated with decreased transactivation function of PPARγ2 and lower body mass index (BMI) [51]. This finding is consistent with reduced adipogenic function of the mutant receptor in 3T3-L1 preadipocytes [52]. However, further studies in various ethnic populations demonstrate that effect of this mutation on body mass is more complex. An association of Ala12 variant with decreased adiposity is confirmed in diabetic, nondiabetic, or healthy subjects [53–58]. In studies involving an African American population and a white American population, this mutation is associated with lower BMI in African Americans and increased BMI in white Americans [59, 60], indicating that same genetic mutation results in different responses in different ethnic groups. PPARγ2^{Pro12Ala} mutation has been also shown to enhance weight loss brought about by exercise in offspring of type 2 diabetic subject [61] or prevent body weight regain after weight loss [62–64]. However, numerous studies suggests an association of Ala12 variant with increased risk of obesity, including studies in ethnic populations of Mexican Americans [65], male Spanish adults [66] or Spanish children and adolescents [67], French [68], male white Italians [69], French Canadians [70], male Brazilians of European descent [71], native Javanese [72], Uygurs, Kazaks, Hans (Chinese) [73], and Greek young girls [74]. This association can also be found in nondiabetic and

nonobese or obese Americans [75], obese Finnish women [76], overweight Korean female subjects [but not in lean female subjects] [77] and in Turkish women with gestational diabetes [78]. In addition, women with the Ala12 allele have also been shown to gain more weight than women with Pro12 allele [79]. Despite these, studies in Germans [80], French [81], Hispanics (Colorado, US) [82], Japanese [83], Koreans [84, 85] and Polish [86] do not show an association between Pro12Ala polymorphism and body fat mass. Meta-analysis of 57 studies on nondiabetic individuals show that Caucasians with the X (Pro or Ala)/12Ala genotype is associated with significantly increased BMI, although no difference can be found in the global population [87]. These results indicate that a mild change in PPARγ2 transcription activity has a significant impact on lipid accumulation in adipose tissue.

It is unclear how a single genetic mutation results in conflict results in different ethnic populations. Given the proadipogenic role of PPARγ, it can be expected that moderate reduction of PPARγ2 transactivation function results in lower BMI in PPAR$\gamma 2^{\text{Pro12Ala}}$ carriers. Heterogeneous effects of this polymorphic mutation on adiposity in association studies clearly show that PPARγ regulation of human adipose tissue physiology is a complex process. Several studies suggest roles of genetic or environmental contexts, such as the character of the diet, in shaping the patterns of associations of Pro12Ala polymorphism with body fat composition in different human populations. In at least two studies, ratio of dietary polyunsaturated fatty acid to saturated fatty acid (P : S ratio) have been shown to significantly affects body mass in Ala12 allele carriers. Thus, intake of a diet with higher P : S ratio results in lower BMI while a food with lower P : S ratio is inversely associated with BMI in human subjects carrying Ala12 allele [88]. Similarly, intake of monounsaturated fatty acid also shows such an effect in Ala12 allele carriers [89]. In another study, total fat and saturated fat intake is positively correlated with body mass change in Pro12 homozygotes while Ala12 allele carriers are protected [70]. In addition, changes in genetic context, such as coexistence of other polymorphisms, may have a significant impact on the effect of Pro12Ala polymorphism on body weight composition, resulting opposite findings mentioned above (flip-flop phenomenon) [90]. For example, either Pro12Ala or G174C (promoter region) of interleukin 6 (IL-6) shows an effect on reducing body fat mass or preventing body weight regain after weight loss and the presence of both variants has an additive effect [54, 62]. On the other hand, subjects bearing both Pro12Ala and Trp64Arg of β_3-adrenergic receptor (β_3-AR^{Trp64Arg}) have increased risk to obesity when compared to those carrying only a single mutation in a case-control study [67] or in a study in dizygotic twins [91], while subjects with the Ala12 allele become more obese only when they also carry the Trp64Arg variant in a Mexican American population [92]. These data suggest complex interactions between genes that both affect lipid metabolism. Yet, there is no study thus far to show that the effect of Pro12Ala polymorphism is negated by mutations in other genes.

3. PPAR$\gamma 2^{\text{Pro12Ala}}$ Regulating Insulin Sensitivity

PPAR$\gamma 2^{\text{Pro12Ala}}$ has also been found to increase insulin sensitivity in middle-aged and elderly Finns [51] and this finding is confirmed by subsequent studies in other populations, assessed by plasma levels of insulin and homeostasis model assessment of insulin resistance (HOMA-IR) [54, 55, 59, 66, 79, 93–95]. In healthy carriers of the Ala12 allele, second-phase insulin secretion in response to free fatty acid infusion or insulin secretion in response to arginine is significantly decreased compared to subjects with Pro12 genotype [96]. Although increased glucose uptake in skeletal muscle is observed only in lean but not in obese subjects in Finns carrying Ala12 allele [97], enhanced insulin sensitivity is observed in obese children [98, 99] as well as in obese adults [100, 101]. Even in diabetic patients, Ala12 allele is associate with lower fasting insulin and increased insulin sensitivity [102], more significant hypoglycemic effect of exercise [103], and increased response to TZD treatment [104]. A population-based study in twins also shows a significant impact of the Ala12 allele on maintaining glucose tolerance and insulin sensitivity [105]. Meta-analysis of such studies confirmed a significantly lower levels of fasting insulin in subjects with the homozygous Ala12Ala genotype compared to the Pro12Pro genotype and significantly greater fasting glucose levels and insulin resistance in obese subjects in the Pro12Pro group [87]. These findings point to a beneficial effect of Ala12 variant on systemic insulin sensitivity.

The effect of PPAR$\gamma 2^{\text{Pro12Ala}}$ polymorphism on insulin sensitivity can be influenced by dietary fatty acids and/or physical activity. Intake of monounsaturated fatty acids is inversely associated with insulin resistance in a Spanish population with Ala12 allele, especially in those with significant obesity [106]. Both dietary P : S ratio and physical activity have been shown to inversely associated with fasting insulin concentration [107]. The effect of dietary P : S ratio on fasting insulin is significant only in physically active, but not in physical inactive subjects carrying Ala12 allele [108]. Ala12 allele also interacts with other genes to influence insulin sensitivity. PPARα Leu162Val allele has been found to be associated with impaired glucose tolerance and this deleterious effect of PPARα mutation is neutralized by the Ala12 variant [109]. Similarly, the Gly > Arg mutation (Gly97Arg) of the insulin receptor substrate 1 (IRS1) is associated with a 15% increased risk of type 2 diabetes, although the difference is not significant [110]. Against this genetic background, insulin sensitivity is almost twice greater in carriers of the 12Ala allele than in subjects with Pro12 allele while no such effect of Ala12 allele can be seen on the Gly97 background [111]. Such a protective effect of Ala12 allele on insulin sensitivity can also be observed in human subjects carrying both the Ala12 allele and the Lys121Gln polymorphism of plasma cell 1 (PC-1) glycoprotein [112]. Subjects bearing PC-$1^{\text{Lys121Gln}}$ variant show higher levels of fasting glucose and decreased insulin sensitivity on Pro12 background, whereas this effect of PC-$1^{\text{Lys121Gln}}$ variant is lost on Ala12 background [113]. These results further support the notion that PPAR$\gamma 2^{\text{Pro12Ala}}$ polymorphism interacts with

other genetic mutations to affect systemic insulin sensitivity and glucose homeostasis.

4. Association of PPAR$\gamma 2^{Pro12Ala}$ with the Risk of Type II Diabetes

A large-scale family-based study shows an association between Pro12Ala mutation and reduced risk of type 2 diabetes (T2D) [110]. A similar result is obtained in twins carrying Ala12 allele [105]. However, further studies clearly show heterogeneous effects of this polymorphism on predicting susceptibility to the risk of diabetes in various populations. Resistance to the risk of diabetes has been found in Ala12 allele carriers compared to Pro12 allele carriers in ethnic populations as diverse as Japanese [114–116], Korean [117], Iranians [118], Scotts [119], Danish [120], Finns [121], French [122], Spanish [106], and American Caucasians [123, 124]. On the other hand, Ala12 allele has also shown to be functional leading to a predisposition to T2D in populations of Germans [125, 126], Finns [127], Italians [128], Dutch [129], US Caucasians [130], French Caucasians [81], British/Irish Caucasians [131], Asian Indians (Sikh) [132], Parkateje Indians [133], and Arabians [134]. Again, no such effect of Ala12 on the risk of type 2 diabetes can be observed in such diverse populations of Italians [135], Tunisians [136], Qatarians [137], Polish [138], and non-Hispanic and Hispanic white women [139]. In spite of such heterogeneity, however, meta-analysis of these studies indicates that Ala12 carriers have an average of 19% reduced risk of T2D compared to Pro12 carriers. BMI seems to be a major factor accountable for the heterogeneous effect of Pro12Ala polymorphism on the risk for T2D since the risk reduction is greater when BMI is lower. Risk reduction is higher in Asians carrying Ala12 allele (35%) than in Northern Americans and Europeans with the Ala12 genotype (18% and 15%, resp.) compared to their own Pro12 allele controls. When adjusted for the BMI of controls, difference between Asians and Europeans is no longer significant. Even among Europeans, Northern Europeans carrying Ala12 allele show significantly reduced risk for T2D (26%) while the risk reduction in Central and Southern Europeans with Ala12 allele is barely significantly (10%) or is not significant at all (0%) [140]. These data suggest a generally beneficial role of Ala12 allele in preventing the pathogenesis of T2D in several populations with lower body fat mass.

While the heterogeneity between Asians and other populations is statistically explained by BMI, this is not the case for the heterogeneity observed in Europeans, indicating that other factors, including different genetic and/or environmental background might cause the heterogeneous Pro12Ala-related T2D risk in Europeans. Indeed, the protective role of Ala12 allele against T2D is considerably affected by dietary lipid levels. In a study in human subjects from Ethiopia, Benin, Ecuador, Italy, and world populations, protection against T2D can be observed mainly in populations where energy from lipids exceeds 30% of total energy intake [141]. However, lipid composition in the diet is a significant determination factor since chronic intake of *trans* fatty acids and saturated fatty acids predispose to increased risk of T2D and impaired fasting glucose in Ala12 carriers than Pro12 carriers [142]. In addition, intrauterine condition may also determine the risk of T2D in later life. A study in Dutch population suggests that subjects bearing Ala12 allele are associated with a higher prevalence of impaired glucose tolerance and T2D when they are prenatally exposed to famine during midgestation [129]. On the other hand, Finns carrying Ala12 allele who have smaller body weight at birth seem to be protected against insulin resistance and T2D [143]. Again, Pro12Ala polymorphism interacts with other genetic mutations to affect the risk of developing diabetes. Subjects with the Ala12Ala allele and Gly972Gly variant of IRS-1 have significantly higher plasma adiponectin levels compared to those with the Pro12Pro and Gly972Gly genotype [144]. In Mexican Americans, subjects with the Ala12 allele become more obese only when they also carry the Trp64Arg of Beta-3 adrenergic receptor (β-3AR^{Trp64Arg}) polymorphism [92]. In a study in dizygotic twin pairs, those with both β-3AR^{Trp64Arg} and PPAR$\gamma 2^{Pro12Ala}$ polymorphisms show greater BMI, waist to hip ratio, percent of body fat, and blood glucose [91]. Such interaction between the two polymorphisms also increases the risk of obesity in children and adolescents [67]. In a family-based study in Chinese and Japanese, subjects with both Ala12 allele and the adiponectin T allele are more insulin sensitive than subjects bearing other combinations of genotypes [145]. Recently, an interaction between Ala12 variant and a single nucleotide polymorphism of PPARδ (rs6902123) has been found to contribute to conversion from impaired glucose tolerance to T2D [121]. These studies again emphasize the importance of taking into account of other gene mutations when determining an effect of Pro12Ala polymorphism on the risk of T2D.

5. Effect on Other Components of Metabolic Syndrome

The Ala12 allele has been shown to be associated with reduced prevalence of essential hypertension in Chinese nonagenarians and centenarian [146]. Ala12 allele carriers also show lower blood pressure than subjects carrying Pro12 allele [120, 147] and the Ala12 allele is associated with lower diastolic blood pressure in male, but not in female subjects with T2D [148]. Furthermore, hypertensive subjects with lower birth weight or shorter length at birth and Pro12Pro variant have raised blood systolic blood pressure [149]. However, others have suggested either a potential contribution of Ala12 variant to hypertension [115] or an association of Ala12 allele with higher diastolic blood pressure in obese patients with T2D [150] while couple of studies fails to show an association between the PPAR$\gamma 2$ variant and hypertension [151, 152].

Triglyceride (TAG) and cholesterol metabolism may be regulated by Pro12Ala mutation. Ala12 allele is inversely associated with blood TAG concentrations in one report [54] while it has also been found to be associated with a trend of an increase in TAG and hyperlipidemia in another [152]. This variant has also been shown to be associated with lower

levels of serum total and nonhigh-density lipoprotein (non-HDL)-cholesterol in a general population [153], lower low-density lipoprotein (LDL)-cholesterol in T2D patients [154], or higher levels of serum HDL-cholesterol in family-based or population-based studies [155, 156]. however, several studies also show an association of Ala12 allele with higher concentration of low-density lipoprotein (LDL)-cholesterol [68, 157] and lower HDL-cholesterol [70]. Interestingly, Pro12Ala mutation interacts with body size at birth to modulate cholesterol metabolism since an association between increased concentration of serum total, LDL- and non-HDL-cholesterol and Ala12 allele can be found only in those who had birth weights below 3 kilograms [158]. In addition, cholesterol metabolism is also affected by genotype-alcohol interaction since Ala12 allele carriers consuming alcohol have higher serum total and HDL cholesterol while the nondrinkers carrying Ala12 allele show lower serum total and HDL cholesterol compared with Pro12 homozygotes [155].

Due to its role in regulating lipid metabolism, Pro12Ala polymorphism may influence risk of cardiovascular complications such as atherosclerosis and coronary artery diseases. Ala12 allele does not seem to affect the risk of acute myocardial infarction, coronary artery disease, and ischemic stroke in healthy subjects [159, 160]. In a population with an increased risk of T2D and cardiovascular disease, however, improvement in flow-mediated vasodilation and reduction of serum C-reactive protein (CRP), a risk factor for cardiovascular disease, are prominent only in Ala12 allele carriers, but not in Pro12 homozygotes [161]. Consistently, Ala12 allele carriers have been found to have lower carotid intima-media thickness [162, 163] and decreased risk of myocardial infarction [164] in T2D patients. Yet again, studies do show that Ala12 allele either is associated with increased risk of myocardial infarction [165, 166], or attenuates the protective effect of polyunsaturated fatty acids on myocardial infarction [167], or confers excess hazard of developing cardiovascular diseases in patients with diabetic nephropathy [168].

As a result of affecting lipid homeostasis and risk of diabetes, Pro12Ala mutation can be expected to influence diabetic complications. Notably, Ala12 allele is associated with decreased risk of developing diabetic nephropathy compared to Pro12 allele in a case-control study [169]. Ala12 allele carriers also have significantly reduced urinary albumin excretion than noncarriers and the reduction becomes even more dramatic along with increased duration of diabetes [154, 170]. Ala12 variant has also been shown to be associated with decreased risk of diabetic retinopathy in T2D patients [171]. These data suggest a protective effect of the Ala12 allele in relation to complications associated with T2D.

6. Effect on Polycystic Ovary Syndrome

Central obesity, insulin resistance, and hyperinsulinemia are typical feature of polycystic ovary syndrome (PCOS) and significant number of PCOS patients show impaired glucose tolerance and are in increased risk of developing T2D [172]. Studies show that frequency of Ala12 allele is significantly reduced in the PCOS group compared with the control group [173, 174]. Moreover, PCOS subjects carrying Ala12 allele show lower levels of free sex hormones (testosterone, androstenedione, and dehydroepiandrosterone sulfate) and reduced luteinizing hormone/follicle-stimulating hormone ratio compared to PCOS subjects carrying Pro12 allele [174]. Insulin sensitivity, evidenced by fasting insulin and HOMA-IR, is also significantly improved in Ala12 allele carriers than in Pro12 allele carriers [174–177]. Even in first-degree relatives of PCOS subjects, distribution of Ala12 Allele is significantly reduced compared to Pro12 allele [178] and fasting insulin and HOMA-IR are lower in first-degree relatives of PCOS subjects with Ala12 variant compared to first-degree relatives of PCOS subjects with Pro12 allele [178].

7. Cellular Mechanism of PPAR$\gamma 2^{Pro12Ala}$ Polymorphism

Since PPARγ2 is expressed only in adipose tissue, how moderate reduction of PPARγ2 activity in adipose tissue influences insulin sensitivity, diabetes, and other metabolic parameters have been studied but not fully elucidated. Given the role of adipose tissue free fatty acids and adipokines in regulating insulin sensitivity, the effect of Pro12Ala polymorphism can be anticipated to be mediated by changes in these factors. Indeed, subjects with Ala12 allele show lower lipoprotein lipase (LPL) activity [179], which may result in decreased breakdown of lipoproteins and hence, reduced plasma FFAs, which is deleterious to insulin action in skeletal muscle [180]. Consistent with this, Ala12 allele carriers have lower plasma FFAs, higher adipose tissue and skeletal muscle blood flow, and greater insulin-mediated postprandial hormone-sensitive lipase suppression along with greater insulin sensitivity [181]. Besides, insulin suppression of lipolysis in adipose tissue is also increased in lean subjects or in T2D patients carrying Ala12 allele than in subjects with Pro12Pro allele [182, 183]. However, long-term inhibition of lipolysis will, in theory, result in increased adiposity (body mass) rather than lean phenotype in Ala12 allele carriers. Indeed, one study suggests there is an association between Ala12 allele and increased body mass [182]. Obviously, this may not be the true mechanism or may not be the only mechanism underlying the effect of Pro12Ala. Adipose-derived cytokines leptin and adiponectin levels have been shown to increase insulin action [184, 185]. Indeed, Ala12 allele is associated with higher plasma levels of leptin in Spanish diabetic women [186]. In comparison, two Japanese population studies show that Ala12 allele carriers have significantly lower plasma levels of adiponectin than Pro12 allele carriers [187, 188] and another two case-control studies in either diabetic patients or women with PCOS fail to find significant change in serum adiponectin levels [189, 190]. Adiponectin does not seem to play a role in increasing insulin sensitivity in Ala12 allele carriers. Finally, recent studies suggest that increased oxidative stress in adipose

tissue is a contributing factor to insulin resistance in obesity [191] and that insulin sensitization by PPARγ agonists is mediated, at least in part, by suppressing oxidative stress in adipose tissue [192]. In adipose tissue-restricted PPARγ heterozygous mice that show reduction of PPARγ in adipose tissue and similarly increased insulin sensitivity as in human subjects carrying Ala12 allele, antioxidant genes are significantly increased; this may be associated with increased resistance to chemical-induced oxidative stress in these animals [193]. Yet, it has not been investigated whether Pro12Ala polymorphism of PPARγ2 is associated with changes in oxidative stress in adipose tissue thus far.

8. PPAR$\gamma 2^{Pro12Ala}$ Polymorphism and Risk of Cancers

PPARγ ligands have been shown to inhibit proliferation of many tumor cells in vitro and PPARγ may also be implicated in tumorigenesis in vivo [194]. Although PPARγ2 is exclusively expressed in adipose tissue, genetic variation of PPARγ2 seems to indirectly affect the risk of several forms of tumors. The most studied thus far is the association between Ala12 allele with the risk of colorectal cancer. The Ala12 variant is inversely associated with incident sporadic colorectal adenoma, and the effect of this mutation is especially pronounced in women and those who do not take nonsteroidal anti-inflammatory drugs [195]. In a case-control study, Ala12 allele, together with high lutein intake, low refinery grain intake and a high prudent diet score, is associated with reduced risk of colon cancer [196]. Interestingly, the same study shows an increased rectal cancer risk in Ala12 carriers [196]. In another case-control study, Pro12Pro genotype is associated with increased risk of colorectal cancer while no such association is observed among Ala12 carriers [197]. In comparison, there is no evidence to show a significant association of Ala12 allele with colorectal cancer in an Indian (Asia) population [198]. In 3 studies related to gastric cancer, Ala12 allele has been found to be associated with increased risk of gastric cancer [199–201] and this effect of PPARγ is probably related to gastric mucosa atrophy and *Helipobacteria pylori* infection since the presence of Ala12 allele does not increase the risk of gastric cancer in *H. pylori*-negative subjects [199]. In two studies on prostate cancer, one study finds a 2-fold greater risk of prostate cancer in Ala12 allele carriers with BMI above 27.2 kg/m^2 compared to those with the Pro12 allele [202] while the other study fails to notice such an association [203]. In addition, a marginally significant increase in the risk of breast cancer is observed in women carrying Ala12 variant [204], but Ala12 allele may decrease the risk of breast cancer associated with alcohol consumption [205]. Finally, Ala12 variant is associated with reduced risk of bladder cancer [206] and renal cell carcinoma [207]. The reason underlying some of the inconsistent findings is unclear, but again may reflect a possibility of gene-gene interaction. In at least one study, Pro12Ala allele interacts with vitamin D receptor (VDR)/bsm/polyA to increase risk of rectal cancer [208].

9. Effect on Aging and Alzheimer Disease

The potential role of genetic variability at Pro/Ala loci of PPARγ2 gene on longevity is studied in a group of centenarians and long-lived men show an increased frequency of Pro/Ala genotype [209]. PPARγ may also be associated with Alzheimer disease (AD) since activation of PPARγ decreases the release of amyloid-β (Aβ), main component of the amyloid plaques associated with AD [210–212]. In line with these observations, a study shows significant overrepresentation of Ala12 allele in octogenarian AD patients, compared to Pro12 allele [213]. However, this result is in contrast with a reported role of Ala12 variant in protecting pathogenesis of AD in female, but not in male subjects in a case-control study [214], while two studies fail to show an association between the Ala12 variant with the genetic risk of AD [215, 216]. Nevertheless, Ala12 allele carriers show an earlier onset of dementia [215], suggesting that Ala12 allele may modify the age of onset in late-onset AD. Ala12 allele carriers also show increased risk of dementia or cognitive impairment without dementia than noncarriers in diabetic patients [217, 218]. It is unclear how PPAR$\gamma 2^{Pro12Ala}$ polymorphism confers such effects on human lifespan or age-related diseases since a change in PPARγ activity by this mutation is supposed to happen only in adipose tissue. Indeed, preliminary studies suggest that the effect of Ala12 allele on human aging may be attributable to decreased IL-6 levels, although there are also reports that healthy elderly have higher levels of IL-6 [219, 220]. In addition, PPAR$\gamma 2^{Pro12Ala}$ polymorphism may affect pathogenesis of AD by modulating cholesterol metabolism since cholesterol levels influence AD pathology [221, 222]. Studies in larger population are required to further elaborate the role of PPAR$\gamma 2^{Pro12Ala}$ polymorphism on blood cholesterol metabolism and AD.

10. Conclusion

Much has been done to evaluate the association between PPAR$\gamma 2^{Pro12Ala}$ polymorphism and body mass, insulin sensitivity, risk of T2D, cancer, and other aspects of human health. However, it is not fully understood how reduction of PPARγ activity in adipose tissue can have such diverse effects on human health. While alteration of fatty acid and cytokine release from adipose tissue may underlie the effect of this mutation on insulin sensitivity and the risk of T2D, it is hard to believe that these factors also account for the effect of Pro12Ala polymorphism on cancer and age-related disease. It is likely that some factors that are overlooked or some unknown factors from adipose tissue may also play a role. Besides, the conflicting results often observed in association studies clearly show the presence of gene-gene interaction. Future association studies should employ a more comprehensive approach, such as linkage disequilibrium or haplotype analyses [223, 224], to examine influence of variants at other genetic loci that may compromise or enhance allelic effect of a genetic polymorphism. PPAR$\gamma 2^{Pro12Ala}$ polymorphism will be a good

model to elucidate how alteration of adipose PPARγ activity affects metabolic program and other aspects of human physiology.

References

[1] D. J. Mangelsdorf, C. Thummel, M. Beato, et al.., "The nuclear receptor superfamily: the second decade," *Cell*, vol. 83, no. 6, pp. 835–839, 1995.

[2] C. K. Glass and S. Ogawa, "Combinatorial roles of nuclear receptors in inflammation and immunity," *Nature Reviews Immunology*, vol. 6, no. 1, pp. 44–55, 2006.

[3] D. J. Mangelsdorf and R. M. Evans, "The RXR heterodimers and orphan receptors," *Cell*, vol. 83, no. 6, pp. 841–850, 1995.

[4] V. Perissi and M. G. Rosenfeld, "Controlling nuclear receptors: the circular logic of cofactor cycles," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 7, pp. 542–554, 2005.

[5] L. Fajas, D. Auboeuf, E. Raspé, et al.., "The organization, promoter analysis, and expression of the human PPARγ gene," *The Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[6] M. E. Greene, B. Blumberg, O. W. McBride, et al.., "Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping," *Gene Expression*, vol. 4, no. 4-5, pp. 281–299, 1995.

[7] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[8] S. A. Kliewer, B. M. Forman, B. Blumberg, et al.., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 15, pp. 7355–7359, 1994.

[9] B. M. Spiegelman, "PPAR-γ: adipogenic regulator and thiazolidinedione receptor," *Diabetes*, vol. 47, no. 4, pp. 507–514, 1998.

[10] Y. Barak, M. C. Nelson, E. S. Ong, et al.., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[11] E. D. Rosen, P. Sarraf, A. E. Troy, et al.., "PPARγ is required for the differentiation of adipose tissue in vivo and in vitro," *Molecular Cell*, vol. 4, no. 4, pp. 611–617, 1999.

[12] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[13] N. Kubota, Y. Terauchi, H. Miki, et al.., "PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance," *Molecular Cell*, vol. 4, no. 4, pp. 597–609, 1999.

[14] W. He, Y. Barak, A. Hevener, et al.., "Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15712–15717, 2003.

[15] T. Imai, R. Takakuwa, S. Marchand, et al.., "Peroxisome proliferator-activated receptor γ is required in mature white and brown adipocytes for their survival in the mouse," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 13, pp. 4543–4547, 2004.

[16] J. R. Jones, C. Barrick, K.-A. Kim, et al.., "Deletion of PPARγ in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 17, pp. 6207–6212, 2005.

[17] L. Nagy, P. Tontonoz, J. G. A. Alvarez, H. Chen, and R. M. Evans, "Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ," *Cell*, vol. 93, no. 2, pp. 229–240, 1998.

[18] P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans, "PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL," *Cell*, vol. 93, no. 2, pp. 241–252, 1998.

[19] A. Chawla, W. A. Boisvert, C.-H. Lee, et al.., "A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis," *Molecular Cell*, vol. 7, no. 1, pp. 161–171, 2001.

[20] K. J. Moore, E. D. Rosen, M. L. Fitzgerald, et al.., "The role of PPAR-γ in macrophage differentiation and cholesterol uptake," *Nature Medicine*, vol. 7, no. 1, pp. 41–47, 2001.

[21] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[22] D. Vats, L. Mukundan, J. I. Odegaard, et al.., "Oxidative metabolism and PGC-1β attenuate macrophage-mediated inflammation," *Cell Metabolism*, vol. 4, no. 1, pp. 13–24, 2006.

[23] A. L. Hevener, J. M. Olefsky, D. Reichart, et al.., "Macrophage PPARγ is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones," *The Journal of Clinical Investigation*, vol. 117, no. 6, pp. 1658–1669, 2007.

[24] J. I. Odegaard, R. R. Ricardo-Gonzalez, M. H. Goforth, C. R. Morel, V. Subramanian, and L. Mukundan, "Macrophage-specific PPARγ controls alternative activation and improves insulin resistance," *Nature*, vol. 447, no. 7148, pp. 1116–1120, 2007.

[25] A. L. Hevener, W. He, Y. Barak, et al.., "Muscle-specific Pparg deletion causes insulin resistance," *Nature Medicine*, vol. 9, no. 12, pp. 1491–1497, 2003.

[26] A. W. Norris, L. Chen, S. J. Fisher, et al.., "Muscle-specific PPARγ-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones," *The Journal of Clinical Investigation*, vol. 112, no. 4, pp. 608–618, 2003.

[27] O. Gavrilova, M. Haluzik, K. Matsusue, et al.., "Liver peroxisome proliferator-activated receptor γ contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass," *The Journal of Biological Chemistry*, vol. 278, no. 36, pp. 34268–34276, 2003.

[28] K. Matsusue, M. Haluzik, G. Lambert, et al.., "Liver-specific disruption of PPARγ in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes," *The Journal of Clinical Investigation*, vol. 111, no. 5, pp. 737–747, 2003.

[29] Y. Wan, L.-W. Chong, and R. M. Evans, "PPAR-γ regulates osteoclastogenesis in mice," *Nature Medicine*, vol. 13, no. 12, pp. 1496–1503, 2007.

[30] G. Ding, M. Fu, Q. Qin, et al.., "Cardiac peroxisome proliferator-activated receptor γ is essential in protecting cardiomyocytes from oxidative damage," *Cardiovascular Research*, vol. 76, no. 2, pp. 269–279, 2007.

[31] S. Z. Duan, C. Y. Ivashchenko, M. W. Russell, D. S. Milstone, and R. M. Mortensen, "Cardiomyocyte-specffic knockout and agonist of peroxisome proliferator-activated receptor-γ both induce cardiac hypertrophy in mice," *Circulation Research*, vol. 97, no. 4, pp. 372–379, 2005.

[32] C. J. Nicol, M. Adachi, T. E. Akiyama, and F. J. Gonzalez, "PPARγ in endothelial cells influences high fat diet-induced hypertension," *American Journal of Hypertension*, vol. 18, no. 4, pp. 549–556, 2005.

[33] R. M. Evans, G. D. Barish, and Y.-X. Wang, "PPARs and the complex journey to obesity," *Nature Medicine*, vol. 10, no. 4, pp. 355–361, 2004.

[34] C. R. Kahn, L. Chen, and S. E. Cohen, "Unraveling the mechanism of action of thiazolidinediones," *The Journal of Clinical Investigation*, vol. 106, no. 11, pp. 1305–1307, 2000.

[35] J. M. Olefsky, "Treatment of insulin resistance with peroxisome proliferator-activated receptor γ agonists," *The Journal of Clinical Investigation*, vol. 106, no. 4, pp. 467–472, 2000.

[36] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *The Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[37] Y. Chung-Jen, B. A. Beamer, C. Negri, et al.., "Molecular scanning of the human peroxisome proliferator activated receptor γ (hPPARγ) gene in diabetic Caucasians: identification of a Pro12Ala PPARγ2 missense mutation," *Biochemical and Biophysical Research Communications*, vol. 241, no. 2, pp. 270–274, 1997.

[38] A. K. Agarwal and A. Garg, "A novel heterozygous mutation in peroxisome proliferator-activated receptor-γ gene in a patient with familial partial lipodystrophy," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 1, pp. 408–411, 2002.

[39] M. Agostini, M. Gurnell, D. B. Savage, et al.., "Tyrosine agonists reverse the molecular defects associated with dominant-negative mutations in human peroxisome proliferator-activated receptor γ," *Endocrinology*, vol. 145, no. 4, pp. 1527–1538, 2004.

[40] M. Agostini, E. Schoenmakers, C. Mitchell, et al.., "Non-DNA binding, dominant-negative, human PPARγ mutations cause lipodystrophic insulin resistance," *Cell Metabolism*, vol. 4, no. 4, pp. 303–311, 2006.

[41] I. Barroso, M. Gurnell, V. E. F. Crowley, et al.., "Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension," *Nature*, vol. 402, no. 6764, pp. 880–883, 1999.

[42] R. A. Hegele, H. Cao, C. Frankowski, S. T. Mathews, and T. Leff, "PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy," *Diabetes*, vol. 51, no. 12, pp. 3586–3590, 2002.

[43] M. Ristow, D. Müller-Wieland, A. Pfeiffer, W. Krone, and C. R. Kahn, "Obesity associated with a mutation in a genetic regulator of adipocyte differentiation," *The New England Journal of Medicine*, vol. 339, no. 14, pp. 953–959, 1998.

[44] D. B. Savage, M. Agostini, I. Barroso, et al.., "Digenic inheritance of severe insulin resistance in a human pedigree," *Nature Genetics*, vol. 31, no. 4, pp. 379–384, 2002.

[45] D. B. Savage, G. D. Tan, C. L. Acerini, et al.., "Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-γ," *Diabetes*, vol. 52, no. 4, pp. 910–917, 2003.

[46] J. P. Berg, "Pluripotent PPARγ polymorphisms," *European Journal of Endocrinology*, vol. 140, no. 4, pp. 293–295, 1999.

[47] M. Gurnell, "PPARγ and metabolism: insights from the study of human genetic variants," *Clinical Endocrinology*, vol. 59, no. 3, pp. 267–277, 2003.

[48] S. Heikkinen, J. Auwerx, and C. A. Argmann, "PPARgamma in human and mouse physiology," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 999–1013, 2007.

[49] Y. Kagawa, Y. Yanagisawa, K. Hasegawa, et al.., "Single nucleotide polymorphisms of thrifty genes for energy metabolism: evolutionary origins and prospects for intervention to prevent obesity-related diseases," *Biochemical and Biophysical Research Communications*, vol. 295, no. 2, pp. 207–222, 2002.

[50] A. Meirhaeghe and P. Amouyel, "Impact of genetic variation of PPARγ in humans," *Molecular Genetics and Metabolism*, vol. 83, no. 1-2, pp. 93–102, 2004.

[51] S. S. Deeb, L. Fajas, M. Nemoto, et al.., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[52] J. Masugi, Y. Tamori, H. Mori, T. Koike, and M. Kasuga, "Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-γ 2 on thiazolidinedione-induced adipogenesis," *Biochemical and Biophysical Research Communications*, vol. 268, no. 1, pp. 178–182, 2000.

[53] A. Doney, B. Fischer, D. Frew, et al.., "Haplotype analysis of the PPARγ Prol 12Ala and CI431T variants reveals opposing associations with body weight," *BMC Genetics*, vol. 3, article 21, pp. 1–8, 2002.

[54] M. Barbieri, M. R. Rizzo, M. Papa, et al.., "Role of interaction between variants in the PPARG and interleukin-6 genes on obesity related metabolic risk factors," *Experimental Gerontology*, vol. 40, no. 7, pp. 599–604, 2005.

[55] C. M. Damcott, S. P. Moffett, E. Feingold, et al.., "Genetic variation in fatty acid-binding protein-4 and peroxisome proliferator-activated receptor γ interactively influence insulin sensitivity and body composition in males," *Metabolism: Clinical and Experimental*, vol. 53, no. 3, pp. 303–309, 2004.

[56] E. L. Rosado, J. Bressan, M. F. Martins, P. R. Cecon, and J. A. Martínez, "Polymorphism in the PPARgamma2 and beta2-adrenergic genes and diet lipid effects on body composition, energy expenditure and eating behavior of obese women," *Appetite*, vol. 49, no. 3, pp. 635–643, 2007.

[57] L. B. Tankó, A. Siddiq, C. Lecoeur, et al.., "ACDC/adiponectin and PPAR-γ gene polymorphisms: implications for features of obesity," *Obesity Research*, vol. 13, no. 12, pp. 2113–2121, 2005.

[58] R. Bouhaha, D. Meyre, H. A. Kamoun, et al.., "Effect of ENPP1/PC-1-K121Q and PPARγ-Pro12Ala polymorphisms on the genetic susceptibility to T2D in the Tunisian population," *Diabetes Research and Clinical Practice*, vol. 81, no. 3, pp. 278–283, 2008.

[59] M. Fornage, D. R. Jacobs Jr., M. W. Steffes, M. D. Gross, M. S. Bray, and P. J. Schreiner, "Inverse effects of the PPARγ2 Pro12Ala polymorphism on measures of adiposity over 15 years in African Americans and whites: the CARDIA study," *Metabolism: Clinical and Experimental*, vol. 54, no. 7, pp. 910–917, 2005.

[60] Q. Wei, D. R. Jacobs Jr., P. J. Schreiner, D. S. Siscovick, M. W. Steffes, and M. Fornage, "Patterns of association between

PPARγ genetic variation and indices of adiposity and insulin action in African-Americans and whites: the CARDIA Study," *Journal of Molecular Medicine*, vol. 84, no. 11, pp. 955–965, 2006.

[61] T. Østergård, J. Ek, Y. Hamid, et al.., "Influence of the PPAR-γ2 Pro12Ala and ACE I/D polymorphisms on insulin sensitivity and training effects in healthy offspring of type 2 diabetic subjects," *Hormone and Metabolic Research*, vol. 37, no. 2, pp. 99–105, 2005.

[62] E. Goyenechea, M. D. Parra, and J. A. Martínez, "Weight regain after slimming induced by an energy-restricted diet depends on interleukin-6 and peroxisome-proliferator-activated-receptor-γ2 gene polymorphisms," *British Journal of Nutrition*, vol. 96, no. 5, pp. 965–972, 2006.

[63] N. Vogels, E. C. Mariman, F. G. Bouwman, A. D. Kester, K. Diepvens, and M. S. Westerterp-Plantenga, "Relation of weight maintenance and dietary restraint to peroxisome proliferator-activated receptor γ2, glucocorticoid receptor, and ciliary neurotrophic factor polymorphisms," *The American Journal of Clinical Nutrition*, vol. 82, no. 4, pp. 740–746, 2005.

[64] K. B. Adamo, R. Dent, C. D. Langefeld, et al.., "Peroxisome proliferator-activated receptor γ 2 and acyl-CoA synthetase 5 polymorphisms influence diet response," *Obesity*, vol. 15, no. 5, pp. 1068–1075, 2007.

[65] S. A. Cole, B. D. Mitchell, W.-C. Hsueh, et al.., "The Pro12Ala variant of peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) is associated with measures of obesity in Mexican Americans," *International Journal of Obesity and Related Metabolic Disorders*, vol. 24, no. 4, pp. 522–524, 2000.

[66] J. L. González Sánchez, M. Serrano Ríos, C. Fernández Pérez, M. Laakso, and M. T. Martínez Larrad, "Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population," *European Journal of Endocrinology*, vol. 147, no. 4, pp. 495–501, 2002.

[67] M. C. Ochoa, A. Marti, C. Azcona, et al.., "Gene-gene interaction between PPARγ2 and ADRβ3 increases obesity risk in children and adolescents," *International Journal of Obesity*, vol. 28, supplement 3, pp. S37–S41, 2004.

[68] A. Meirhaeghe, M. W. T. Tanck, L. Fajas, et al.., "Study of a new PPARγ2 promoter polymorphism and haplotype analysis in a French population," *Molecular Genetics and Metabolism*, vol. 85, no. 2, pp. 140–148, 2005.

[69] E. Morini, V. Tassi, D. Capponi, et al.., "Interaction between PPARγ2 variants and gender on the modulation of body weight," *Obesity*, vol. 16, no. 6, pp. 1467–1470, 2008.

[70] J. Robitaille, J.-P. Després, L. Pérusse, and M.-C. Vohl, "The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Québec Family Study," *Clinical Genetics*, vol. 63, no. 2, pp. 109–116, 2003.

[71] V. S. Mattevi, V. M. Zembrzuski, and M. H. Hutz, "Effects of a PPARG gene variant on obesity characteristics in Brazil," *Brazilian Journal of Medical and Biological Research*, vol. 40, no. 7, pp. 927–932, 2007.

[72] C. W. Danawati, M. Nagata, H. Moriyama, et al.., "A possible association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor γ2 gene with obesity in native Javanese in Indonesia," *Diabetes/Metabolism Research and Reviews*, vol. 21, no. 5, pp. 465–469, 2005.

[73] L.-L. Li, X.-L. Ma, J.-X. Ran, et al.., "Genetic polymorphism of peroxisome proliferator-activated receptor-γ2 Pro12Ala on ethnic susceptibility to diabetes in Uygur, Kazak and Han subjects," *Clinical and Experimental Pharmacology and Physiology*, vol. 35, no. 2, pp. 187–191, 2008.

[74] V. Lagou, R. A. Scott, Y. Manios, et al.., "Impact of peroxisome proliferator-activated receptors γ and δ on adiposity in toddlers and preschoolers in the GENESIS study," *Obesity*, vol. 16, no. 4, pp. 913–918, 2008.

[75] B. A. Beamer, C.-J. Yen, R. E. Andersen, et al.., "Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-γ2 gene with obesity in two Caucasian populations," *Diabetes*, vol. 47, no. 11, pp. 1806–1808, 1998.

[76] R. Valve, K. Sivenius, R. Miettinen, et al.., "Two polymorphisms in the peroxisome proliferator-activated receptor-γ gene are associated with severe overweight among obese women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 84, no. 10, pp. 3708–3712, 1999.

[77] K. S. Kim, S. M. Choi, S. U. Shin, H. S. Yang, and Y. Yoon, "Effects of peroxisome proliferator-activated receptor-γ2 Pro12Ala polymorphism on body fat distribution in female Korean subjects," *Metabolism: Clinical and Experimental*, vol. 53, no. 12, pp. 1538–1543, 2004.

[78] E. C. Tok, D. Ertunc, O. Bilgin, E. M. Erdal, M. Kaplanoglu, and S. Dilek, "PPAR-γ2 Pro12Ala polymorphism is associated with weight gain in women with gestational diabetes mellitus," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 129, no. 1, pp. 25–30, 2006.

[79] B. J. Nicklas, E. F. C. van Rossum, D. M. Berman, A. S. Ryan, K. E. Dennis, and A. R. Shuldiner, "Genetic variation in the peroxisome proliferator-activated receptor-γ2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain," *Diabetes*, vol. 50, no. 9, pp. 2172–2176, 2001.

[80] D. Evans, W. A. Mann, J. de Heer, et al.., "Variation in the gene for human peroxisome proliferator activated receptor γ (PPARγ) does not play a major role in the development of morbid obesity," *International Journal of Obesity*, vol. 24, no. 5, pp. 647–651, 2000.

[81] M. Ghoussaini, D. Meyre, S. Lobbens, et al.., "Implication of the Pro12Ala polymorphism of the *PPAR-gamma 2* gene in type 2 diabetes and obesity in the French population," *BMC Medical Genetics*, vol. 6, article 11, pp. 1–8, 2005.

[82] T. L. Nelson, T. E. Fingerlin, L. Moss, M. M. Barmada, R. E. Ferrell, and J. M. Norris, "The PPARγ Pro12Ala polymorphism is not associated with body mass index or waist circumference among hispanics from Colorado," *Annals of Nutrition and Metabolism*, vol. 51, no. 3, pp. 252–257, 2007.

[83] Y. Mori, H. Kim-Motoyama, T. Katakura, et al.., "Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor γ2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men," *Biochemical and Biophysical Research Communications*, vol. 251, no. 1, pp. 195–198, 1998.

[84] E. Y. Oh, K. M. Min, J. H. Chung, et al.., "Significance of Pro^{12}Ala mutation in peroxisome proliferator-activated receptor-γ2 in Korean diabetic and obese subjects," *The Journal of Clinical Endocrinology & Metabolism*, vol. 85, no. 5, pp. 1801–1804, 2000.

[85] K. Kim, S. Lee, and R. J. Valentine, "Association of Pro12Ala polymorphism in the peroxisome proliferative-activated receptor γ2 gene with obesity and hypertension in Korean women," *Journal of Nutritional Science and Vitaminology*, vol. 53, no. 3, pp. 239–246, 2007.

[86] A. Stefanski, L. Majkowska, A. Ciechanowicz, et al.., "Lack of association between the Pro12Ala polymorphism in PPAR-γ2 gene and body weight changes, insulin resistance and chronic diabetic complications in obese patients with type 2 diabetes," *Archives of Medical Research*, vol. 37, no. 6, pp. 736–743, 2006.

[87] A. Tönjes, M. Scholz, M. Loeffler, and M. Stumvoll, "Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor γ with pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals," *Diabetes Care*, vol. 29, no. 11, pp. 2489–2497, 2006.

[88] J. Luan, P. O. Browne, A.-H. Harding, et al.., "Evidence for gene-nutrient interaction at the PPARγ locus," *Diabetes*, vol. 50, no. 3, pp. 686–689, 2001.

[89] A. Memisoglu, F. B. Hu, S. E. Hankinson, et al.., "Interaction between a peroxisome proliferator-activated receptor γ gene polymorphism and dietary fat intake in relation to body mass," *Human Molecular Genetics*, vol. 12, no. 22, pp. 2923–2929, 2003.

[90] P.-I. Lin, J. M. Vance, M. A. Pericak-Vance, and E. R. Martin, "No gene is an island: the flip-flop phenomenon," *American Journal of Human Genetics*, vol. 80, no. 3, pp. 531–538, 2007.

[91] T.-J. Chen, C.-Y. Ji, X.-Y. Zheng, and Y.-H. Hu, "Association of β3 adrenergic receptor and peroxisome proliferator-activated receptor gamma 2 polymorphisms with insulin sensitivity: a twin study," *Biomedical and Environmental Sciences*, vol. 20, no. 2, pp. 99–105, 2007.

[92] W.-C. Hsueh, S. A. Cole, A. R. Shuldiner, et al.., "Interactions between variants in the β3-adrenergic receptor and peroxisome proliferator-activated receptor-γ2 genes and obesity," *Diabetes Care*, vol. 24, no. 4, pp. 672–677, 2001.

[93] A. Fritsche, A. Madaus, O. Tschritter, et al.., "Pro12Ala polymorphism in peroxisome proliferator-activated receptorγ_2 (PPARγ_2): beta-cell function and insulin sensitivity," *Deutsche Medizinische Wochenschrift*, vol. 126, no. 20, pp. 580–584, 2001.

[94] U. Helwig, D. Rubin, J. Kiosz, et al.., "The minor allele of the PPARγ2 Pro12Ala polymorphism is associated with lower postprandial TAG and insulin levels in non-obese healthy men," *British Journal of Nutrition*, vol. 97, no. 5, pp. 847–854, 2007.

[95] T. Kahara, T. Takamura, T. Hayakawa, et al.., "PPARγ gene polymorphism is associated with exercise-mediated changes of insulin resistance in healthy men," *Metabolism: Clinical and Experimental*, vol. 52, no. 2, pp. 209–212, 2003.

[96] N. Stefan, A. Fritsche, H. Häring, and M. Stumvoll, "Effect of experimental elevation of free fatty acids on insulin secretion and insulin sensitivity in healthy carriers of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 gene," *Diabetes*, vol. 50, no. 5, pp. 1143–1148, 2001.

[97] M. Vänttinen, P. Nuutila, J. Pihlajamäki, et al.., "The effect of the Ala12 allele of the peroxisome proliferator-activated receptor-γ2 gene on skeletal muscle glucose uptake depends on obesity: a positron emission tomography study," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 7, pp. 4249–4254, 2005.

[98] R. Buzzetti, A. Petrone, A. M. Caiazzo, et al.., "PPAR-γ2 Pro12Ala variant is associated with greater insulin sensitivity in childhood obesity," *Pediatric Research*, vol. 57, no. 1, pp. 138–140, 2005.

[99] S. Scaglioni, E. Verduci, M. Salvioni, et al.., "PPAR-γ2 Pro12Ala variant, insulin resistance and plasma long-chain polyunsaturated fatty acids in childhood obesity," *Pediatric Research*, vol. 60, no. 4, pp. 485–489, 2006.

[100] R. Buzzetti, A. Petrone, M. C. Ribaudo, et al.., "The common PPAR-γ2 Pro12Ala variant is associated with greater insulin sensitivity," *European Journal of Human Genetics*, vol. 12, no. 12, pp. 1050–1054, 2004.

[101] M. Koch, K. Rett, E. Maerker, et al.., "The PPARγ2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects," *Diabetologia*, vol. 42, no. 6, pp. 758–762, 1999.

[102] V. Tavares, R. D. C. Hirata, A. C. Rodrigues, et al.., "Association between Pro12Ala polymorphism of the PPAR-γ2 gene and insulin sensitivity in Brazilian patients with type-2 diabetes mellitus," *Diabetes, Obesity and Metabolism*, vol. 7, no. 5, pp. 605–611, 2005.

[103] K. B. Adamo, R. J. Sigal, K. Williams, G. Kenny, D. Prud'homme, and F. Tesson, "Influence of Pro^{12}Ala peroxisome proliferator-activated receptor γ2 polymorphism on glucose response to exercise training in type 2 diabetes," *Diabetologia*, vol. 48, no. 8, pp. 1503–1509, 2005.

[104] E. S. Kang, S. Y. Park, H. J. Kim, et al.., "Effects of Pro12Ala polymorphism of peroxisome proliferator-activated receptor γ2 gene on rosiglitazone response in type 2 diabetes," *Clinical Pharmacology & Therapeutics*, vol. 78, no. 2, pp. 202–208, 2005.

[105] P. Poulsen, G. Andersen, M. Fenger, et al.., "Impact of two common polymorphisms in the PPARγ gene on glucose tolerance and plasma insulin profiles in monozygotic and dizygotic twins: thrifty genotype, thrifty phenotype, or both?" *Diabetes*, vol. 52, no. 1, pp. 194–198, 2003.

[106] F. Soriguer, S. Morcillo, F. Cardona, et al.., "Pro12Ala polymorphism of the PPARG2 gene is associated with type 2 diabetes mellitus and peripheral insulin sensitivity in a population with a high intake of oleic acid," *The Journal of Nutrition*, vol. 136, no. 9, pp. 2325–2330, 2006.

[107] A.-H. Harding, D. E. M. Williams, S. H. J. Hennings, J. Mitchell, and N. J. Wareham, "Is the association between dietary fat intake and insulin resistance modified by physical activity?" *Metabolism: Clinical and Experimental*, vol. 50, no. 10, pp. 1186–1192, 2001.

[108] P. W. Franks, J. Luan, P. O. Browne, et al.., "Does peroxisome proliferator-activated receptor γ genotype (Pro12ala) modify the association of physical activity and dietary fat with fasting insulin level?" *Metabolism: Clinical and Experimental*, vol. 53, no. 1, pp. 11–16, 2004.

[109] Y. Bossé, S. J. Weisnagel, C. Bouchard, J.-P. Després, L. Pérusse, and M.-C. Vohl, "Combined effects of PPARγ_2 P12A and PPARα L162V polymorphisms on glucose and insulin homeostasis: the Québec Family Study," *Journal of Human Genetics*, vol. 48, no. 12, pp. 614–621, 2003.

[110] D. Altshuler, J. N. Hirschhorn, M. Klannemark, et al.., "The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes," *Nature Genetics*, vol. 26, no. 1, pp. 76–80, 2000.

[111] M. Stumvoll, N. Stefan, A. Fritsche, et al.., "Interaction effect between common polymorphisms in PPARγ 2 (Pro12Ala) and insulin receptor substrate 1 (Gly972Arg) on insulin sensitivity," *Journal of Molecular Medicine*, vol. 80, no. 1, pp. 33–38, 2002.

[112] R. Baratta, R. Di Paola, D. Spampinato, et al.., "Evidence for genetic epistasis in human insulin resistance: the combined

effect of PC-1 (K121Q) and PPARγ2 (P12A) polymorphisms," *Journal of Molecular Medicine*, vol. 81, no. 11, pp. 718–723, 2003.

[113] A. Pizzuti, L. Frittitta, A. Argiolas, et al.., "A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance," *Diabetes*, vol. 48, no. 9, pp. 1881–1884, 1999.

[114] K. Hara, T. Okada, K. Tobe, et al.., "The Pro12Ala polymorphism in PPAR γ2 may confer resistance to type 2 diabetes," *Biochemical and Biophysical Research Communications*, vol. 271, no. 1, pp. 212–216, 2000.

[115] M. Horiki, H. Ikegami, T. Fujisawa, et al.., "Association of Pro12Ala polymorphism of PPARγ gene with insulin resistance and related diseases," *Diabetes Research and Clinical Practice*, vol. 66, supplement 1, pp. S63–S67, 2004.

[116] H. Mori, H. Ikegami, Y. Kawaguchi, et al.., "The Pro^{12} → Ala substitution in PPAR-γ 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*, vol. 50, no. 4, pp. 891–894, 2001.

[117] M. K. Moon, Y. M. Cho, H. S. Jung, et al.., "Genetic polymorphisms in peroxisome proliferator-activated receptor γ are associated with type 2 diabetes mellitus and obesity in the Korean population," *Diabetic Medicine*, vol. 22, no. 9, pp. 1161–1166, 2005.

[118] R. Meshkani, M. Taghikhani, B. Larijani, et al.., "Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 (PPARγ-2) gene is associated with greater insulin sensitivity and decreased risk of type 2 diabetes in an Iranian population," *Clinical Chemistry and Laboratory Medicine*, vol. 45, no. 4, pp. 477–482, 2007.

[119] A. S. F. Doney, B. Fischer, J. E. Cecil, et al.., "Association of the Pro12Ala and C1431T variants of *PPARG* and their haplotypes with susceptibility to type 2 diabetes," *Diabetologia*, vol. 47, no. 3, pp. 555–558, 2004.

[120] L. Frederiksen, K. Brødbæk, M. Fenger, et al.., "Studies of the Pro12Ala polymorphism of the PPAR-γ gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 8, pp. 3989–3992, 2002.

[121] L. Andrulionytè, J. Zacharova, J.-L. Chiasson, and M. Laakso, "Common polymorphisms of the PPAR-γ2 (Pro12Ala) and PGC-1α (Gly482Ser) genes are associated with the conversion from impaired glucose tolerance to type 2 diabetes in the STOP-NIDDM trial," *Diabetologia*, vol. 47, no. 12, pp. 2176–2184, 2004.

[122] R. Jaziri, S. Lobbens, R. Aubert, et al.., "The *PPARG* Pro12Ala polymorphism is associated with a decreased risk of developing hyperglycemia over 6 years and combines with the effect of the *APM1* G-11391A single nucleotide polymorphism: the data from an epidemiological study on the insulin resistance syndrome (DESIR) study," *Diabetes*, vol. 55, no. 4, pp. 1157–1162, 2006.

[123] A. Memisoglu, F. B. Hu, S. E. Hankinson, et al.., "Prospective study of the association between the proline to alanine codon 12 polymorphism in the PPARγ gene and type 2 diabetes," *Diabetes Care*, vol. 26, no. 10, pp. 2915–2917, 2003.

[124] V. Radha, K. S. Vimaleswaran, H. N. S. Babu, et al.., "Role of genetic polymorphism peroxisome proliferator-activated receptor-γ2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: evidence for heterogeneity," *Diabetes Care*, vol. 29, no. 5, pp. 1046–1051, 2006.

[125] D. Evans, J. de Heer, C. Hagemann, et al.., "Association between the P12A and c1431t polymorphisms in the peroxisome proliferator activated receptor γ (PPARγ) gene and type 2 diabetes," *Experimental and Clinical Endocrinology and Diabetes*, vol. 109, no. 3, pp. 151–154, 2001.

[126] C. Herder, W. Rathmann, K. Strassburger, et al.., "Variants of the *PPARG, IGF2BP2, CDKAL1, HHEX*, and *TCF7L2* genes confer risk of type 2 diabetes independently of BMI in the German KORA studies," *Hormone and Metabolic Research*, vol. 40, no. 10, pp. 722–726, 2008.

[127] T. O. Kilpeläinen, T. A. Lakka, D. E. Laaksonen, et al.., "SNPs in PPARG associate with type 2 diabetes and interact with physical activity," *Medicine and Science in Sports and Exercise*, vol. 40, no. 1, pp. 25–33, 2008.

[128] M. Fiorito, I. Torrente, S. De Cosmo, et al.., "Interaction of *DIO2* T92A and PPARγ2 P12A polymorphisms in the modulation of metabolic syndrome," *Obesity*, vol. 15, no. 12, pp. 2889–2895, 2007.

[129] S. R. de Rooij, R. C. Painter, D. I. W. Phillips, et al.., "The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 gene on glucose/insulin metabolism interact with prenatal exposure to famine," *Diabetes Care*, vol. 29, no. 5, pp. 1052–1057, 2006.

[130] J. C. Florez, K. A. Jablonski, M. W. Sun, et al.., "Effects of the type 2 diabetes-associated PPARG P12A polymorphism on progression to diabetes and response to troglitazone," *The Journal of Clinical Endocrinology & Metabolism*, vol. 92, no. 4, pp. 1502–1509, 2007.

[131] E. Zeggini, J. R. C. Parkinson, S. Halford, et al.., "Examining the relationships between the Pro12Ala variant in *PPARG* and type 2 diabetes-related traits in UK samples," *Diabetic Medicine*, vol. 22, no. 12, pp. 1696–1700, 2005.

[132] D. K. Sanghera, L. Ortega, S. Han, et al.., "Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: *PPARG2* (*Pro12Ala*), *IGF2BP2, TCF7L2* and *FTO* variants confer a significant risk," *BMC Medical Genetics*, vol. 9, article 59, pp. 1–9, 2008.

[133] J. P. B. Vieira-Filho, A. F. Reis, T. S. Kasamatsu, et al.., "Influence of the polymorphisms Tpr64Arg in the β3-adrenergic receptor gene and Pro12Ala in the PPARγ2 gene on metabolic syndrome-related phenotypes in an indigenous population of the Brazilian Amazon," *Diabetes Care*, vol. 27, no. 2, pp. 621–622, 2004.

[134] S. M. Wakil, K. Al-Rubeaan, O. Alsmadi, et al.., "The peroxisome proliferator-activated receptor-γ2 P12A polymorphism and type 2 diabetes in an Arab population," *Diabetes Care*, vol. 29, no. 1, pp. 171–172, 2006.

[135] F. P. Mancini, O. Vaccaro, L. Sabatino, et al.., "Pro12Ala substitution in the peroxisome proliferator-activated receptor-γ2 is not associated with type 2 diabetes," *Diabetes*, vol. 48, no. 7, pp. 1466–1468, 1999.

[136] K. Z. Bouassida, L. Chouchane, K. Jellouli, et al.., "The peroxisome proliterator activated receptorγ2 (PPARγ2) Pro12Ala variant: lack of association with type 2 diabetes in obese and non obese Tunisian patients," *Diabetes and Metabolism*, vol. 31, no. 2, pp. 119–123, 2005.

[137] R. Badii, A. Bener, M. Zirie, et al.., "Lack of association between the Pro12Ala polymorphism of the PPAR-γ2 gene and type 2 diabetes mellitus in the Qatari consanguineous population," *Acta Diabetologica*, vol. 45, no. 1, pp. 15–21, 2008.

[138] M. T. Malecki, J. Frey, T. Klupa, et al.., "The Pro12Ala polymorphism of PPARγ2 gene and susceptibility to type 2 diabetes mellitus in a Polish population," *Diabetes Research and Clinical Practice*, vol. 62, no. 2, pp. 105–111, 2003.

[139] S. P. Moffett, E. Feingold, M. M. Barmada, et al.., "The C161 → T polymorphism in peroxisome proliferator-activated receptor gamma, but not P12A, is associated with insulin resistance in Hispanic and non-Hispanic white women: evidence for another functional variant in peroxisome proliferator-activated receptor gamma," *Metabolism: Clinical and Experimental*, vol. 54, no. 11, pp. 1552–1556, 2005.

[140] O. Ludovico, F. Pellegrini, R. Di Paola, et al.., "Heterogeneous effect of peroxisome proliferator-activated receptor γ2 *Ala12* variant on type 2 diabetes risk," *Obesity*, vol. 15, no. 5, pp. 1076–1081, 2007.

[141] R. Scacchi, A. Pinto, O. Rickards, et al.., "An analysis of peroxisome proliferator-activated receptor gamma (PPAR-γ2) Pro12Ala polymorphism distribution and prevalence of type 2 diabetes mellitus (T2DM) in world populations in relation to dietary habits," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 17, no. 9, pp. 632–641, 2007.

[142] R. E. Pisabarro, C. Sanguinetti, M. Stoll, and D. Prendez, "High incidence of type 2 diabetes in peroxisome proliferator-activated receptor γ2 Pro12Ala carriers exposed to a high chronic intake of *trans* fatty acids and saturated fatty acids," *Diabetes Care*, vol. 27, no. 9, pp. 2251–2252, 2004.

[143] J. G. Eriksson, "Gene polymorphisms, size at birth, and the development of hypertension and type 2 diabetes," *The Journal of Nutrition*, vol. 137, no. 4, pp. 1063–1065, 2007.

[144] F. Mousavinasab, T. Tähtinen, J. Jokelainen, et al.., "Effect of the Pro12Ala polymorphism of the PPARγ2 gene on serum adiponectin changes," *Endocrine*, vol. 27, no. 3, pp. 307–309, 2005.

[145] W.-S. Yang, C. A. Hsiung, L.-T. Ho, et al.., "Genetic epistasis of adiponectin and PPARγ2 genotypes in modulation of insulin sensitivity: a family-based association study," *Diabetologia*, vol. 46, no. 7, pp. 977–983, 2003.

[146] Z. Lu, B. Dong, X. Mo, et al.., "Pro12Ala polymorphism in PPAR γ 2 associated with essential hypertension in Chinese nonagenarians/centenarians," *Experimental Gerontology*, vol. 43, no. 12, pp. 1108–1113, 2008.

[147] F. J. Rodríguez-Esparragón, J. C. Rodríguez-Pérez, A. Macías-Reyes, and F. Alamo-Santana, "Peroxisome proliferator-activated receptor-gamma2-Pro12Ala and endothelial nitric oxide synthase-4*a/b* gene polymorphisms are associated with essential hypertension," *Journal of Hypertension*, vol. 21, no. 9, pp. 1649–1655, 2003.

[148] C. J. Östgren, U. Lindblad, O. Melander, A. Melander, L. Groop, and L. Råstam, "Peroxisome proliferator-activated receptor-γ*Pro12Ala* polymorphism and the association with blood pressure in type 2 diabetes: Skaraborg Hypertension and Diabetes Project," *Journal of Hypertension*, vol. 21, no. 9, pp. 1657–1662, 2003.

[149] H. Ylihärsilä, J. G. Eriksson, T. Forsén, et al.., "Interactions between peroxisome proliferator-activated receptor-γ2 polymorphisms and size at birth on blood pressure and the use of antihypertensive medication," *Journal of Hypertension*, vol. 22, no. 7, pp. 1283–1287, 2004.

[150] A. Stefański, L. Majkowska, A. Ciechanowicz, et al.., "Association between the Pro12Ala variant of the peroxisome proliferator-activated receptor-gamma2 gene and increased 24-h diastolic blood pressure in obese patients with type II diabetes," *Journal of Human Hypertension*, vol. 20, no. 9, pp. 684–692, 2006.

[151] I. Gouni-Berthold, E. Giannakidou, D. Müller-Wieland, et al.., "Peroxisome proliferator-activated receptor-γ2 Pro12Ala and endothelial nitric oxide synthase-4a/b gene polymorphisms are not associated with hypertension in diabetes mellitus type 2," *Journal of Hypertension*, vol. 23, no. 2, pp. 301–308, 2005.

[152] M. M. Swarbrick, C. M. Chapman, B. M. McQuillan, J. Hung, P. L. Thompson, and J. P. Beilby, "A Pro12Ala polymorphism in the human peroxisome proliferator-activated receptor-γ2 is associated with combined hyperlipidaemia in obesity," *European Journal of Endocrinology*, vol. 144, no. 3, pp. 277–282, 2001.

[153] E. Iwata, H. Matsuda, T. Fukuda, et al.., "Mutations of the peroxisome proliferator-activated receptor γ (PPARγ) gene in a Japanese population: the Pro12Ala mutation in PPARγ2 is associated with lower concentrations of serum total and non-HDL cholesterol," *Diabetologia*, vol. 44, no. 10, pp. 1354–1355, 2001.

[154] R. L. Pollex, M. Mamakeesick, B. Zinman, S. B. Harris, R. A. Hegele, and A. J. G. Hanley, "Peroxisome proliferator-activated receptor γ polymorphism Pro12Ala is associated with nephropathy in type 2 diabetes," *Journal of Diabetes and Its Complications*, vol. 21, no. 3, pp. 166–171, 2007.

[155] S.-M. Brand-Herrmann, T. Kuznetsova, A. Wiechert, et al.., "Alcohol intake modulates the genetic association between HDL cholesterol and the PPARγ2 Pro12Ala polymorphism," *Journal of Lipid Research*, vol. 46, no. 5, pp. 913–919, 2005.

[156] E. S. Tai, D. Corella, M. Deurenberg-Yap, et al.., "Differential effects of the C1431T and Pro12Ala PPARγ gene variants on plasma lipids and diabetes risk in an Asian population," *Journal of Lipid Research*, vol. 45, no. 4, pp. 674–685, 2004.

[157] T. Hamada, K. Kotani, K. Tsuzaki, et al.., "Association of Pro12Ala polymorphism in the peroxisome proliferator-activated receptor γ2 gene with small dense low-density lipoprotein in the general population," *Metabolism: Clinical and Experimental*, vol. 56, no. 10, pp. 1345–1349, 2007.

[158] J. Eriksson, V. Lindi, M. Uusitupa, et al.., "The effects of the Pro12Ala polymorphism of the *PPARγ*-2 gene on lipid metabolism interact with body size at birth," *Clinical Genetics*, vol. 64, no. 4, pp. 366–370, 2003.

[159] E. J. Rhee, C. H. Kwon, W. Y. Lee, et al.., "No association of Pro12Ala polymorphism of PPAR-γ gene with coronary artery disease in Korean subjects," *Circulation Journal*, vol. 71, no. 3, pp. 338–342, 2007.

[160] M. H. Zafarmand, Y. T. van der Schouw, D. E. Grobbee, P. W. de Leeuw, and M. L. Bots, "Peroxisome proliferator-activated receptor gamma-2 P12A polymorphism and risk of acute myocardial infarction, coronary heart disease and ischemic stroke: a case-cohort study and meta-analyses," *Vascular Health and Risk Management*, vol. 4, no. 2, pp. 427–436, 2008.

[161] K. Rittig, C. Thamer, F. Machicao, et al.., "The Pro12Ala polymorphism in *PPARG2* increases the effectiveness of primary prevention of cardiovascular disease by a lifestyle intervention," *Diabetologia*, vol. 50, no. 6, pp. 1345–1347, 2007.

[162] K. Z. Al-Shali, A. A. House, A. J. G. Hanley, et al.., "Genetic variation in *PPARG* encoding peroxisome proliferator-activated receptor γ associated with carotid atherosclerosis," *Stroke*, vol. 35, no. 9, pp. 2036–2040, 2004.

[163] E. Iwata, I. Yamamoto, T. Motomura, et al.., "The association of *Pro12Ala* polymorphism in *PPARγ2* with lower carotid

artery IMT in Japanese," *Diabetes Research and Clinical Practice*, vol. 62, no. 1, pp. 55–59, 2003.

[164] A. S. F. Doney, B. Fischer, G. Leese, A. D. Morris, and C. N. A. Palmer, "Cardiovascular risk in type 2 diabetes is associated with variation at the *PPARG* locus: a Go-DARTS study," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 12, pp. 2403–2407, 2004.

[165] L. Li, L.-X. Cheng, R. Nsenga, M.-A. He, and T.-C. Wu, "Association between Pro12Ala polymorphism of peroxisome proliferator-activated receptor-gamma 2 and myocardial infarction in the Chinese han population," *Clinical Cardiology*, vol. 29, no. 7, pp. 300–304, 2006.

[166] T. Pischon, J. K. Pai, J. E. Manson, et al.., "Peroxisome proliferator-activated receptor-γ2 P12A polymorphism and risk of coronary heart disease in US men and women," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 8, pp. 1654–1658, 2005.

[167] E. A. Ruiz-Narváez, P. Kraft, and H. Campos, "Ala12 variant of the peroxisome proliferator-activated receptor-γ gene (*PPARG*) is associated with higher polyunsaturated fat in adipose tissue and attenuates the protective effect of polyunsaturated fat intake on the risk of myocardial infarction," *The American Journal of Clinical Nutrition*, vol. 86, no. 4, pp. 1238–1242, 2007.

[168] C.-C. Szeto, K.-M. Chow, P. Y.-K. Poon, B. C.-H. Kwan, and P. K.-T. Li, "Peroxisome proliferator-activated receptor-gamma gene polymorphism and risk of cardiovascular disease in patients with diabetic nephropathy," *American Journal of Nephrology*, vol. 28, no. 5, pp. 715–722, 2008.

[169] M. L. Caramori, L. H. Canani, L. A. Costa, and J. L. Gross, "The human peroxisome proliferator-activated receptor γ2 (PPARγ2) Pro12Ala polymorphism is associated with decreased risk of diabetic nephropathy in patients with type 2 diabetes," *Diabetes*, vol. 52, no. 12, pp. 3010–3013, 2003.

[170] S.-M. Herrmann, J. Ringel, J.-G. Wang, J. A. Staessen, and E. Brand, "Peroxisome proliferator-activated receptor-γ2 polymorphism Pro12Ala is associated with nephropathy in type 2 diabetes: the Berlin Diabetes Mellitus (BeDiaM) Study," *Diabetes*, vol. 51, no. 8, pp. 2653–2657, 2002.

[171] M. T. Malecki, K. Cyganek, B. Mirkiewicz-Sieradzka, et al.., "Alanine variant of the Pro12Ala polymorphism of the PPARγ gene might be associated with decreased risk of diabetic retinopathy in type 2 diabetes," *Diabetes Research and Clinical Practice*, vol. 80, no. 1, pp. 139–145, 2008.

[172] R. S. Legro, A. R. Kunselman, W. C. Dodson, and A. Dunaif, "Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 84, no. 1, pp. 165–169, 1999.

[173] S. Korhonen, S. Heinonen, M. Hiltunen, et al.., "Polymorphism in the peroxisome proliferator-activated receptor-γ gene in women with polycystic ovary syndrome," *Human Reproduction*, vol. 18, no. 3, pp. 540–543, 2003.

[174] M. Yilmaz, M. A. Ergün, A. Karakoç, E. Yurtçu, N. Çakir, and M. Arslan, "Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ gene in women with polycystic ovary syndrome," *Gynecological Endocrinology*, vol. 22, no. 6, pp. 336–342, 2006.

[175] S. Hahn, A. Fingerhut, U. Khomtsiv, et al.., "The peroxisome proliferator activated receptor gamma Pro12Ala polymorphism is associated with a lower hirsutism score and increased insulin sensitivity in women with polycystic ovary syndrome," *Clinical Endocrinology*, vol. 62, no. 5, pp. 573–579, 2005.

[176] M. Hara, S. Y. Alcoser, A. Qaadir, K. K. Beiswenger, N. J. Cox, and D. A. Ehrmann, "Insulin resistance is attenuated in women with polycystic ovary syndrome with the $Pro^{12}Ala$ polymorphism in the PPARγ gene," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 2, pp. 772–775, 2002.

[177] E. C. Tok, A. Aktas, D. Ertunc, E. M. Erdal, and S. Dilek, "Evaluation of glucose metabolism and reproductive hormones in polycystic ovary syndrome on the basis of peroxisome proliferator-activated receptor (PPAR)-γ2 Pro12Ala genotype," *Human Reproduction*, vol. 20, no. 6, pp. 1590–1595, 2005.

[178] M. Yilmaz, M. A. Ergün, A. Karakoç, et al.., "Pro12Ala polymorphism of the *peroxisome proliferator-activated receptor-γ* gene in first-degree relatives of subjects with polycystic ovary syndrome," *Gynecological Endocrinology*, vol. 21, no. 4, pp. 206–210, 2005.

[179] J. Schneider, J. Kreuzer, A. Hamann, P. P. Nawroth, and K. A. Dugi, "The proline 12 alanine substitution in the peroxisome proliferator-activated receptor-γ2 gene is associated with lower lipoprotein lipase activity in vivo," *Diabetes*, vol. 51, no. 3, pp. 867–870, 2002.

[180] J. Delarue and C. Magnan, "Free fatty acids and insulin resistance," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 10, no. 2, pp. 142–148, 2007.

[181] G. D. Tan, M. J. Neville, E. Liverani, et al.., "The in vivo effects of the Pro12Ala PPARγ2 polymorphism on adipose tissue NEFA metabolism: the first use of the Oxford Biobank," *Diabetologia*, vol. 49, no. 1, pp. 158–168, 2006.

[182] M. Stumvoll and H. Häring, "Reduced lipolysis as possible cause for greater weight gain in subjects with the *Pro12Ala* polymorphism in PPARγ_2?" *Diabetologia*, vol. 45, no. 1, pp. 152–153, 2002.

[183] M. Stumvoll, H. G. Wahl, K. Löblein, et al.., "Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-γ_2 gene is associated with increased antilipolytic insulin sensitivity," *Diabetes*, vol. 50, no. 4, pp. 876–881, 2001.

[184] I. Shimomura, R. E. Hammer, S. Ikemoto, M. S. Brown, and J. L. Goldstein, "Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy," *Nature*, vol. 401, no. 6748, pp. 73–76, 1999.

[185] T. Yamauchi, J. Kamon, Y. Minokoshi, et al.., "Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase," *Nature Medicine*, vol. 8, no. 11, pp. 1288–1295, 2002.

[186] I. Simón, J. Vendrell, C. Gutiérrez, et al.., "Pro12Ala substitution in the peroxisome proliferator-activated receptor-gamma is associated with increased leptin levels in women with type-2 diabetes mellitus," *Hormone Research*, vol. 58, no. 3, pp. 143–149, 2002.

[187] N. Takata, T. Awata, K. Inukai, et al.., "Pro12Ala substitution in peroxisome proliferator-activated receptorγ2 is associated with low adiponectin concentrations in young Japanese men," *Metabolism: Clinical and Experimental*, vol. 53, no. 12, pp. 1548–1551, 2004.

[188] Y. Yamamoto, H. Hirose, K. Miyashita, et al.., "PPARγ_2 gene Pro12Ala polymorphism may influence serum level of an adipocyte-derived protein, adiponectin, in the Japanese population," *Metabolism: Clinical and Experimental*, vol. 51, no. 11, pp. 1407–1409, 2002.

[189] F. Orio Jr., S. Palomba, T. Cascella, et al.., "Lack of an association between peroxisome proliferator-activated receptor-γ gene Pro12Ala polymorphism and adiponectin levels in the polycystic ovary syndrome," *The Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 10, pp. 5110–5115, 2004.

[190] V. Radha, K. S. Vimaleswaran, S. Babu, et al.., "Lack of association between serum adiponectin levels and the Pro12Ala polymorphism in Asian Indians," *Diabetic Medicine*, vol. 24, no. 4, pp. 398–402, 2007.

[191] S. Furukawa, T. Fujita, M. Shimabukuro, et al.., "Increased oxidative stress in obesity and its impact on metabolic syndrome," *The Journal of Clinical Investigation*, vol. 114, no. 12, pp. 1752–1761, 2004.

[192] N. Houstis, E. D. Rosen, and E. S. Lander, "Reactive oxygen species have a causal role in multiple forms of insulin resistance," *Nature*, vol. 440, no. 7086, pp. 944–948, 2006.

[193] W. Luo, J. Cao, J. Li, and W. He, "Adipose tissue-specific PPARγ deficiency increases resistance to oxidative stress," *Experimental Gerontology*, vol. 43, no. 3, pp. 154–163, 2008.

[194] P. Tontonoz and B. M. Spiegelman, "Fat and beyond: the diverse biology of PPARγ," *Annual Review of Biochemistry*, vol. 77, pp. 289–312, 2008.

[195] Z. Gong, D. Xie, Z. Deng, et al.., "The PPARγ Pro12Ala polymorphism and risk for incident sporadic colorectal adenomas," *Carcinogenesis*, vol. 26, no. 3, pp. 579–585, 2005.

[196] M. A. Murtaugh, K. Ma, B. J. Caan, et al.., "Interactions of peroxisome proliferator-activated receptor γ and diet in etiology of colorectal cancer," *Cancer Epidemiology Biomarkers & Prevention*, vol. 14, no. 5, pp. 1224–1229, 2005.

[197] G. Theodoropoulos, I. Papaconstantinou, E. Felekouras, et al.., "Relation between common polymorphisms in genes related to inflammatory response and colarectal cancer," *World Journal of Gastroenterology*, vol. 12, no. 31, pp. 5037–5043, 2006.

[198] J. Jiang, V. Gajalakshmi, J. Wang, et al.., "Influence of the C161T but not Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma on colorectal cancer in an Indian population," *Cancer Science*, vol. 96, no. 8, pp. 507–512, 2005.

[199] S.-Y. Liao, Z.-R. Zeng, W. K. Leung, et al.., "Peroxisome proliferator-activated receptor-gamma Pro12Ala polymorphism, *Helicobacter pylori* infection and non-cardia gastric carcinoma in Chinese," *Alimentary Pharmacology & Therapeutics*, vol. 23, no. 2, pp. 289–294, 2006.

[200] K. N. Prasad, A. Saxena, U. C. Ghoshal, M. R. Bhagat, and N. Krishnani, "Analysis of Pro12Ala *PPAR gamma* polymorphism and *Helicobacter pylori* infection in gastric adenocarcinoma and peptic ulcer disease," *Annals of Oncology*, vol. 19, no. 7, pp. 1299–1303, 2008.

[201] T. Tahara, T. Arisawa, T. Shibata, et al.., "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," *Digestive Diseases and Sciences*, vol. 53, no. 3, pp. 614–621, 2008.

[202] J. M. Zmuda, F. Modugno, J. L. Weissfeld, et al.., "Peroxisome proliferator-activated receptor-γ polymorphism, body mass and prostate cancer risk: evidence for gene-environment interaction," *Oncology*, vol. 70, no. 3, pp. 185–189, 2006.

[203] D. Paltoo, K. Woodson, P. Taylor, D. Albanes, J. Virtamo, and J. Tangrea, "*Pro12Ala* polymorphism in the peroxisome proliferator-activated receptor-gamma (PPAR-γ) gene and risk of prostate cancer among men in a large cancer prevention study," *Cancer Letters*, vol. 191, no. 1, pp. 67–74, 2003.

[204] Y. Wang, M. L. McCullough, V. L. Stevens, et al.., "Nested case-control study of energy regulation candidate gene single nucleotide polymorphisms and breast cancer," *Anticancer Research*, vol. 27, no. 1B, pp. 589–593, 2007.

[205] U. Vogel, J. Christensen, B. A. Nexø, H. Wallin, S. Friis, and A. Tjønneland, "Peroxisome profilerator-activated receptorγ2 Pro^{12}Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes," *Carcinogenesis*, vol. 28, no. 2, pp. 427–434, 2007.

[206] D. Leibovici, H. B. Grossman, C. P. Dinney, et al.., "Polymorphisms in inflammation genes and bladder cancer: from initiation to recurrence, progression, and survival," *Journal of Clinical Oncology*, vol. 23, no. 24, pp. 5746–5756, 2005.

[207] W. M. Smith, X.-P. Zhou, K. Kurose, et al.., "Opposite association of two *PPARG* variants with cancer: overrepresentation of H449H in endometrial carcinoma cases and underrepresentation of P12A in renal cell carcinoma cases," *Human Genetics*, vol. 109, no. 2, pp. 146–151, 2001.

[208] M. L. Slattery, C. Sweeney, M. Murtaugh, et al.., "Associations between vitamin D, vitamin D receptor gene and the androgen receptor gene with colon and rectal cancer," *International Journal of Cancer*, vol. 118, no. 12, pp. 3140–3146, 2006.

[209] M. Barbieri, M. Bonafè, M. R. Rizzo, et al.., "Gender specific association of genetic variation in peroxisome proliferator-activated receptor (PPAR)γ-2 with longevity," *Experimental Gerontology*, vol. 39, no. 7, pp. 1095–1100, 2004.

[210] I. E. Camacho, L. Serneels, K. Spittaels, P. Merchiers, D. Dominguez, and B. De Strooper, "Peroxisome proliferator-activated receptor γ induces a clearance mechanism for the amyloid-β peptide," *The Journal of Neuroscience*, vol. 24, no. 48, pp. 10908–10917, 2004.

[211] C. d'Abramo, S. Massone, J.-M. Zingg, et al.., "Role of peroxisome proliferator-activated receptor γ in amyloid precursor protein processing and amyloid β-mediated cell death," *Biochemical Journal*, vol. 391, no. 3, pp. 693–698, 2005.

[212] M. Sastre, I. Dewachter, S. Rossner, et al.., "Nonsteroidal anti-inflammatory drugs repress β-secretase gene promoter activity by the activation of PPARγ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 2, pp. 443–448, 2006.

[213] R. Scacchi, A. Pinto, G. Gambina, A. Rosano, and R. M. Corbo, "The peroxisome proliferator-activated receptor gamma (PPAR-γ2) Pro12Ala polymorphism is associated with higher risk for Alzheimer's disease in octogenarians," *Brain Research*, vol. 1139, no. 1, pp. 1–5, 2007.

[214] G. Hamilton, P. Proitsi, L. Jehu, et al.., "Candidate gene association study of insulin signaling genes and Alzheimer's disease: evidence for *SOS2*, *PCK1*, and *PPARγ* as susceptibility loci," *American Journal of Medical Genetics Part B*, vol. 144, no. 4, pp. 508–516, 2007.

[215] A. M. Koivisto, S. Helisalmi, J. Pihlajamäki, et al.., "Association analysis of peroxisome proliferator-activated receptor gamma polymorphisms and late onset al.zheimer's disease in the Finnish population," *Dementia and Geriatric Cognitive Disorders*, vol. 22, no. 5-6, pp. 449–453, 2006.

[216] S. Sauder, H. Kölsch, D. Lütjohann, et al.., "Influence of peroxisome proliferator-activated receptor γ gene polymorphism on 24S-hydroxycholesterol levels in Alzheimer's patients," *Journal of Neural Transmission*, vol. 112, no. 10, pp. 1381–1389, 2005.

[217] W. Johnson, S. E. Harris, J. M. Starr, L. J. Whalley, and I. J. Deary, "*PPARG* Pro12Ala genotype and risk of cognitive decline in elders? Maybe with diabetes," *Neuroscience Letters*, vol. 434, no. 1, pp. 50–55, 2008.

[218] N. A. West, M. N. Haan, and H. Morgenstern, "The PPAR-gamma Pro12Ala polymorphism and risk of cognitive impairment in a longitudinal study," *Neurobiology of Aging*. In press.

[219] U. Fagiolo, A. Cossarizza, E. Scala, et al.., "Increased cytokine production in mononuclear cells of healthy elderly people," *European Journal of Immunology*, vol. 23, no. 9, pp. 2375–2378, 1993.

[220] J. Myśliwska, E. Bryl, J. Foerster, and A. Myśliwski, "Increase of interleukin 6 and decrease of interleukin 2 production during the ageing process are influenced by the health status," *Mechanisms of Ageing and Development*, vol. 100, no. 3, pp. 313–328, 1998.

[221] P. Pregelj, "Involvement of cholesterol in the pathogenesis of Alzheimer's disease: role of statins," *Psychiatria Danubina*, vol. 20, no. 2, pp. 162–167, 2008.

[222] P. C. Reid, Y. Urano, T. Kodama, and T. Hamakubo, "Alzheimer's disease: cholesterol, membrane rafts, isoprenoids and statins," *Journal of Cellular and Molecular Medicine*, vol. 11, no. 3, pp. 383–392, 2007.

[223] C. S. Carlson, M. A. Eberle, M. J. Rieder, Q. Yi, L. Kruglyak, and D. A. Nickerson, "Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium," *The American Journal of Human Genetics*, vol. 74, no. 1, pp. 106–120, 2004.

[224] D. C. Crawford, C. S. Carlson, M. J. Rieder, et al.., "Haplotype diversity across 100 candidate genes for inflammation, lipid metabolism, and blood pressure regulation in two populations," *The American Journal of Human Genetics*, vol. 74, no. 4, pp. 610–622, 2004.

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 369602, 5 pages
doi:10.1155/2009/369602

Research Article

Differences in Transcriptional Activation by the Two Allelic (L162V Polymorphic) Variants of PPARα after Omega-3 Fatty Acids Treatment

Iwona Rudkowska,[1,2,3] Mélanie Verreault,[4,5] Olivier Barbier,[4,5] and Marie-Claude Vohl[1,2,3]

[1] *Lipid Research Center, CHUL Research Center, QC, Canada G1V 4G2*
[2] *Department of Food Science and Nutrition, Laval University, QC, Canada G1V 0A6*
[3] *Nutraceuticals and Functional Foods Institute (INAF), Laval University, QC, Canada G1V 0A6*
[4] *Laboratory of Molecular Pharmacology, Oncology and Genomic Research Center, CHUL Research Center, QC, Canada G1V 4G2*
[5] *Faculty of Pharmacy, Laval University, QC, Canada G1V 0A6*

Correspondence should be addressed to Marie-Claude Vohl, marie-claude.vohl@crchul.ulaval.ca

Received 22 August 2008; Revised 26 November 2008; Accepted 21 December 2008

Recommended by Mostafa Badr

Omega-3 fatty acids (FAs) have the potential to regulate gene expression via the peroxisome proliferator-activated receptor α (PPARα); therefore, genetic variations in this gene may impact its transcriptional activity on target genes. It is hypothesized that the transcriptional activity by wild-type L162-PPARα is enhanced to a greater extent than the mutated variant (V162-PPARα) in the presence of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or a mixture of EPA:DHA. To examine the functional difference of the two allelic variants on receptor activity, transient co-transfections were performed in human hepatoma HepG2 cells activated with EPA, DHA and EPA:DHA mixtures. Results indicate that the addition of EPA or DHA demonstrate potential to increase the transcriptional activity by PPARα with respect to basal level in both variants. Yet, the EPA:DHA mixtures enhanced the transcriptional activity to a greater extent than individual FAs indicating possible additive effects of EPA and DHA. Additionally, the V162 allelic form of PPARα demonstrated consistently lower transcriptional activation when incubated with EPA, DHA or EPA:DHA mixtures than, the wild-type variant. In conclusion, both allelic variants of the PPARα L162V are activated by omega-3 FAs; however, the V162 allelic form displays a lower transcriptional activity than the wild-type variant.

1. Introduction

Higher intake of long-chain n-3 fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been recommended to decrease plasma triglyceride (TG) levels. Conventionally, the mechanism of action after n-3 FAs intake focused on plasma membrane fluidity; however, recently the emphasis shifted to regulation of gene expression [1]. In particular, FAs and their derivatives are physiological ligands of peroxisome proliferator-activated receptor α (PPARα). As such they activate the PPARα-retinoid X receptor (RXR) heterodimer-dependent gene transcription by binding to the peroxisome proliferator response elements (PPRE) in the promoter region of target genes [2]. Target genes include lipoprotein lipase (LPL) involved in plasma TG clearance [2].

Several polymorphisms within the PPARα gene and the encoded proteins have been identified including L162V and V227A, which are the most common PPARα polymorphisms reported [3]. Of particular interest, the PPARα L162V polymorphism has been associated with obesity indices and plasma lipid levels in numerous studies [4–8]. Additionally, Robitaille et al. in 2004 found that the interaction between the PPARα L162V polymorphism and fat intake estimated from a food frequency questionnaire (FFQ) explains a significant percentage of the variance observed in waist girth in a sample of 260 French-Canadians [9]. Tai et al. in 2005 [10] also established that the effect of the PPARα L162V polymorphism on plasma TG and apolipoprotein (apo)-CIII concentrations depends on the dietary polyunsaturated FA (PUFA), with a high intake triggering lower TG in carriers of the V162-PPARα variant. Finally, Paradis et al. in 2002 [11]

demonstrated that the interindividual variations in total cholesterol, apo A-I, and cholesterol concentrations in small low-density lipoprotein (LDL) particles observed after modification of the polyunsaturated/saturated FA ratio of the diet is partly attributable to the PPARα L162V polymorphism. Clearly, both epidemiological and interventional studies demonstrate a relation between the PPARα L162V polymorphisms, metabolic parameters, and FAs intake; yet, only two functional studies examined the receptor activity of the L162V polymorphic variants activated with synthetic agonists-fibrates [4, 12]. It was demonstrated that the effect of the L162V polymorphic variants on the transcriptional activation was associated with the concentration of the ligand to which it is exposed [12].

For that reason, the aim of this functional study was to determine whether the transcriptional activity by the wild-type variant, L162-PPARα, is enhanced in the presence of natural PPARα agonists-omega-3 FAs, mimicking the action of synthetic PPARα agonists, comparatively to the variant, V162-PPARα.

2. Laboratory Methods

2.1. Plasmid Construction. The wild-type L162-PPAR expression plasmid (pSG5-hPPARα vector) was a kind gift from Pr. B. Staels (Unité INSERM 545, Institut Pasteur de Lille, France). The pSG5-mRXRα plasmid was described previously [13]. The V162-PPARα expression plasmid was derived from the wild-type, through site-directed mutagenesis (QuickChange site-directed mutagenesis kit, Stratagene, La Jolla, Calif, USA) using the 5′-CGATTTCAC-AAGTGC**G**TTTCTGTCGGGATG-3′ oligonucleotide (the nucleotide in boldface type denotes the C → G change mutated base). Variant cDNAs were directly sequenced to confirm that no spurious base changes have been introduced during the procedure. As a response element representative of the vast variety of human PPREs, we choose the consensus artificial direct repeat (DR)1 sequence for analyzing the functional consequences of L162V variation on PPARα activation by omega-3 FAs. Thus, a reporter plasmid (DR1x6-PPRE) was generated by cloning in front of the thymidine kinase promoter-driven luciferase reporter gene (TKpGL3 vector), six copies of the 5′-AAAACTAGGTCAAAGGTCACGG-3′ sequence where underlined nucleotides correspond to the direct repeat of the AGGTCA hexamer.

2.2. Transient Transfection and n-3 Fatty Acids Activation. Human hepatoma HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented by 10% fetal bovine serum (FBS), 1% of streptomycin penicillin, 1% of sodium pyruvate and 1% of glutamine. HepG2 cells were plated at a density of 75×10^3 cells/well of 24-well plates which were then transfected using the ExGen reagent (Invitrogen, Burlington, Canada) with 50 ng of the DR1x6-PPRE reporter plasmid, 10 ng of the PPARα (wild-type or mutant) and *RXR* expression plasmids and 30 ng of the pRL-NULL expression vector (Promega) for 6 hours 37°C. All samples were complemented with pBS-SK+ plasmid (Stratagene) to an identical amount (500 ng/well). Similar experiments were performed with a negative control consisting of the empty TK-pGL3-basic plasmid (Promega). After transfection, cells were cultured in DMEM supplemented by 0.2% FBS for 24 hours to strengthen cell membrane before addition of FAs. Afterwards, cells were transactivated for 24 hours in absence or presence of omega-3 FAs in concentrations varying between 1–15 μM to reflect biological plasma or red blood cells concentration of FAs [14]. Cells were treated with either solvent (dimethyl sulfoxide (DMSO), 0.01% final concentration), or treatments of EPA and/or DHA (Sigma-Aldrich, Oakville, ON, Canada). Briefly, pure EPA or DHA was dissolved by serial dilution to 1, 5, 10, and 15 μM in DMSO. For mixtures of 5:5, 15:5, and 5:15 μM EPA:DHA, the dissolved individual omega-3 FAs at appropriate concentrations were mixed together. The luciferase activity was quantified with a luminometer (Bertholus, LB956V) and expressed as fold induction in the presence of variable doses of omega-3 FAs over control. Ciprofibrate (250 μM) (Sigma-Aldrich) was used as a positive control of induction. The assays were performed in triplicates. The experiment was conducted in duplicate.

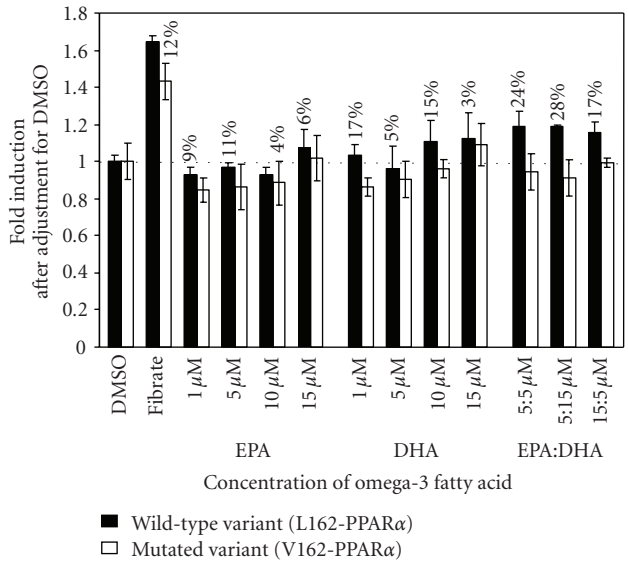
2.3. Data Analysis. Firefly luciferase activities were normalized with the corresponding Renilla luciferase reporter activity as internal control. Fold induction was calculated by taking the control DMSO (Sigma-Aldrich) as baseline.

3. Results

Transient transfection assays in human hepatoma HepG2 cells were done to compare L162-PPARα to V162-PPARα transcriptional activity. In sum, two independent transients' transfection assays were performed with similar results for transcriptional activity. The V162-PPARα variant showed similar basal transcriptional activity after treatment with DMSO compared with L162-PPARα on the DR1x6-PPRE. For positive control, the presence of the PPAR synthetic ligand, ciprofibrate, showed enhanced transactivation activity in V162-PPARα compared with L162-PPARα (Figure 1). Most importantly, the results from this functional study demonstrate that increase in activity in the V162-PPARα variant did not reach the same level of extent of transcriptional activity as the L162-PPARα variant in all replicates and doses of omega-3 FAs.

In more details, the addition of 5 and 15 μM EPA resulted in an increased in activity with respect to basal level of EPA of 1 μM in L162-PPARα variant, yet only 15 μM EPA resulted in a slight increase in transcriptional rate compared to DMSO (Figure 1). In the same way, the addition of 5, 10, and 15 μM EPA resulted in an higher activity, with respect to basal level in V162-PPARα variant (Figure 1). Nevertheless, transcriptional activity by the L162-PPARα variant compared to V162-PPARα variant was 9%, 11%, 4% and 6% consistently greater with 1, 5, 10, and 15 μM of EPA (Figure 1) representing functional differences between the variants.

Similarly, the addition of DHA enhanced transcriptional activity at most concentrations in both the L162-PPARα and V162-PPARα variant compared to basal level of DHA (Figure 1). However, only 10 or 15 μM of DHA



Mean±SD
Percentage difference between variants
Two independent experiments were done with similar results

Figure 1: Transcriptional activity by L162-PPARα and V162-PPARα in HepG2 cells supplemented with EPA, DHA, and mixtures of EPA:DHA. The DR1-PPRE-TKpGL3 reporter construct (100 ng) was cotransfected with the pRL-NULL plasmid (30 ng) in HepG2 cells in presence of 10 ng pSG5-hPPARα wild-type (black bars) or mutated (white bars) and pSG5-mRXRα (10 ng) plasmids. Cells were subsequently treated or not with ciprofibrate (250 μM) or varying concentrations and mixtures of EPA and/or DHA for 24 hours. Values were normalized to internal Renilla luciferase activity as described in materials and methods and expressed as fold-induction relative to the control (TK-pGL3) set at 1. Values are representative of 2 independent experiments realized in triplicates.

in the L162-PPARα increased activity compared to DMSO. Likewise, the addition of 1, 5, 10, or 15 μM of DHA increased to a greater extent the transcriptional activity by the L162-PPARα variant compared to the V162-PPARα variant (17%, 5%, 15%, and 3%; resp.) (Figure 1).

In addition, EPA:DHA mixtures tested showed a marked increase in transcriptional activity that was higher with respect to individual FA transcriptional activity or basal activity (Figure 1). Again in the V162-PPARα variant, the ratios of EPA:DHA increased the receptor activity but to a lesser degree than in L162-PPARα (Figure 1). The disparities in transcriptional activity between the L162-PPARα and V162-PPARα variants were even more important: 24%, 28%, and 17% for 5:5, 5:15 and 15:5 μM EPA:DHA ratios, respectively. Overall, even if the individual FAs show a smaller transcriptional activity by PPARα with a larger standard deviation, this transcriptional activity is consistently lower in the V162-PPARα than L162-PPARα. Further, this information is supported by the results of the mixtures of EPA:DHA, where there is clearly an increased transcriptional activity and this effect is of lesser magnitude in V162-PPARα than L162-PPARα.

4. Discussion

The present study represents the first examination of the variation in transcriptional activity after omega-3 FA activation in the L162V polymorphic variant. Overall, the use of natural PPARα agonists, such as omega-3 FAs, may influence the activation of PPARα at higher doses. Nevertheless, differences exist in the rates of transcriptional activity by the V162-PPARα and the L162-PPARα variant of the PPARα L162V polymorphism. In addition, the additive effects of EPA and DHA mixtures on transcription rates may reveal supplementary benefits compared to the individual omega-3 FAs.

The results clearly reveal that the V162-PPARα has lower transcriptional activity than the L162-PPARα. Previous research has demonstrated the impact of PPARα on the clearance of TG-rich lipoproteins in humans after treatment with PPARα agonist, fibrates [15]. The plasma TG lowering effect of fibrates can be duplicated in animal studies [16]. In contrast, plasma TGs are elevated in animals lacking PPARα [17]. This data suggest that PPARα adjusts LPL-dependent TG lypolysis by altering expression of pro- and antilipolytic factors [18]. Thus, the current results demonstrate that individuals carrying a V162-PPARα variant may potentially have elevated TG levels due to lower transcription rate of target genes, such as *LPL*. These in vitro results support the numerous human studies in which the PPARα L162V polymorphism exhibited associations with total cholesterol, LDL-cholesterol, apo B, TG, and high-density lipoproteins (HDL)-cholesterol [4–9]. In general, from the current and previous human studies, the V162 allele appears to be associated with a more harmful lipid profile potentially due to a lower transcription rate of target genes with PPREs.

The improve transactivation of both allelic variants following an omega-3 FA activation reveals the importance of stratifying individuals according to their dietary fat intakes including omega-3 FA to demonstrate the influence of PPARα L162V polymorphism on lipid parameters and other metabolic factors. Since the mutation is located in the DNA binding domain, this single nucleotide polymorphism is thought to have an impact on the receptor's ability to bind DNA [12]. While receptors coregulators (i.e., coactivators and corepressors) generally interact with the ligand binding domain of nuclear receptors [19], we cannot exclude that the L162V amino acid substitution affects the PPARα's ability to adequately separate from cytoplasmic corepressor, transit to the nuclei and/or recruit coactivators, as it was demonstrated for the V227A variant of this receptor [20]. To the best of our knowledge functional studies have never been performed for the PPARα L162V mutation, and the mechanisms at the basis of differential omega-3 FAs-dependent activation of the wild-type and mutated receptors remain to be elucidated. Yet, it appears that the V162-PPARα has the potential to reach comparable transcription rates as L162-PPARα with higher intakes of individual or mixtures of omega-3 FAs. Therefore, the influence of the L162V polymorphic variant may be more apparent in individuals who consume a lower intake of omega-3 FAs. These results are in accordance with previous human studies [10, 11, 21] which examined the effect of the PPARα L162V polymorphism in relation to diet.

These previous researchers determined that a high intake of dietary PUFA can lower TG in carriers of the V162-PPARα allele [10, 11] due to higher n-3 FA intakes that may lead to increased activation of PPARα. Finally, a recent study by Caron-Dorval et al. in 2008 [21] demonstrated that plasma TG levels decreased similarly between a group of 28 young men with or without the L162V polymorphism after an intense omega-3 FA supplementation for 4 weeks. These results confirm that dietary modifications including higher amounts of EPA and DHA, which activate PPARs to a greater level, may be an effective method in reducing metabolic risk in those with high-risk allele, such as V162. However, this point requires further investigation to ascertain a precise nutritional recommendation.

An additional purpose of this study was to determine whether EPA, DHA, and combinations of EPA:DHA have differential roles in transcriptional activity. Most studies regarding the effects of n-3 PUFA on blood lipids were conducted with fish oils that contain a mixture of EPA and DHA [22, 23]. Yet, a number of studies have been conducted with EPA and DHA individually. In vitro [24] and animal [25–28] studies suggest that EPA rather than DHA may be a hypotriglyceridemic agent. However, divergent findings have been reported in human studies [29, 30]. Results from the current study with individual FAs indicate that a higher dose of either EPA or DHA can increase transcriptional rate of target genes. However, our results demonstrated that DHA may have a slightly higher transcriptional activity than EPA. A recent study by Sanderson et al. in 2008 [31] showed that DHA behaved as a highly potent inducer of PPARα dependent gene expression compared to other FAs, although they did not examine the effects of EPA or mixtures of these FAs. On the other hand, investigators who examined the effects of oleic acid, EPA, and DHA on intestinal gene expression in mice identified 19, 46, and 41 genes, respectively, that were activated with these fatty acids versus 74 genes with the PPARα agonist [32]. In addition in the current study, all tested concentrations of combination of EPA and DHA induced slightly higher transcription rates than individual FAs. However, it still remains unclear whether EPA and DHA have similar TG lowering potential. Further studies are needed to determine whether EPA and DHA in combination, as they are found naturally in fish oils, have an additive effects on gene expression rates; hence, potentially reducing TG concentrations to a greater extent than individual FAs.

In conclusion, these results indicate that the V162-PPARα variant has lower transcriptional activity than L162-PPARα variant in response to omega-3 FAs; therefore, clearly demonstrating that a nutrient-gene interaction exists between PPARα L162V polymorphism and omega-3 FAs. Further studies are needed to confirm whether this difference in transcriptional activity by PPARα is translated into differences in gene expression levels of physiological target genes. Overall, the functional understanding of omega-3 FAs in relation to PPARα L162V genotypes may allow more targeted individualized dietary advice to maximising the benefit gained by the individual.

Acknowledgments

This study was supported by Grant no. MOP-200609 from the Canadian Institutes of Health Research (CIHR). Authors thank Pr. B. Staels for providing the pSG5-hPPAR construct.

References

[1] S. A. Kliewer, S. S. Sundseth, S. A. Jones, et al., "Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 9, pp. 4318–4323, 1997.

[2] S. Kersten, "Peroxisome proliferator activated receptors and lipoprotein metabolism," *PPAR Research*, vol. 2008, Article ID 132960, 11 pages, 2008.

[3] E. L. Yong, J. Li, and M. H. Liu, "Single gene contributions: genetic variants of peroxisome proliferator-activated receptor (isoforms α, β/δ and γ) and mechanisms of dyslipidemias," *Current Opinion in Lipidology*, vol. 19, no. 2, pp. 106–112, 2008.

[4] D. M. Flavell, I. Pineda Torra, Y. Jamshidi, et al., "Variation in the PPARα gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects," *Diabetologia*, vol. 43, no. 5, pp. 673–680, 2000.

[5] E. S. Tai, S. Demissie, L. A. Cupples, et al., "Association between the PPARA L162V polymorphism and plasma lipid levels: the Framingham Offspring Study," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 5, pp. 805–810, 2002.

[6] T. Tanaka, J. M. Ordovas, J. Delgado-Lista, et al., "Peroxisome proliferator-activated receptor α polymorphisms and postprandial lipemia in healthy men," *Journal of Lipid Research*, vol. 48, no. 6, pp. 1402–1408, 2007.

[7] J. Uthurralt, H. Gordish-Dressman, M. Bradbury, et al., "PPARα L162V underlies variation in serum triglycerides and subcutaneous fat volume in young males," *BMC Medical Genetics*, vol. 8, article 55, pp. 1–11, 2007.

[8] T. Sparsø, M. S. Hussain, G. Andersen, et al., "Relationships between the functional PPARα Leu162Val polymorphism and obesity, type 2 diabetes, dyslipidaemia, and related quantitative traits in studies of 5799 middle-aged white people," *Molecular Genetics and Metabolism*, vol. 90, no. 2, pp. 205–209, 2007.

[9] J. Robitaille, C. Brouillette, A. Houde, et al., "Association between the PPARα-L162V polymorphism and components of the metabolic syndrome," *Journal of Human Genetics*, vol. 49, no. 9, pp. 482–489, 2004.

[10] E. S. Tai, D. Corella, S. Demissie, et al., "Polyunsaturated fatty acids interact with the PPARA-L162V polymorphism to affect plasma triglyceride and apolipoprotein C-III concentrations in the Framingham Heart Study," *Journal of Nutrition*, vol. 135, no. 3, pp. 397–403, 2005.

[11] A.-M. Paradis, B. Fontaine-Bisson, Y. Bossé, et al., "The peroxisome proliferator-activated receptor α Leu162Val polymorphism influences the metabolic response to a dietary intervention altering fatty acid proportions in healthy men," *American Journal of Clinical Nutrition*, vol. 81, no. 2, pp. 523–530, 2005.

[12] A. Sapone, J. M. Peters, S. Sakai, et al., "The human peroxisome proliferator-activated receptor α gene: identification and functional characterization of two natural allelic variants," *Pharmacogenetics*, vol. 10, no. 4, pp. 321–333, 2000.

[13] N. Vu-Dac, K. Schoonjans, V. Kosykh, et al., "Retinoids increase human apolipoprotein A-II expression through activation of the retinoid X receptor but not the retinoic acid receptor," *Molecular and Cellular Biology*, vol. 16, no. 7, pp. 3350–3360, 1996.

[14] E. Jungling and H. Kammermeier, "A one-vial method for routine extraction and quantification of free fatty acids in blood and tissue by HPLC," *Analytical Biochemistry*, vol. 171, no. 1, pp. 150–157, 1988.

[15] H. S. Simpson, C. M. Williamson, T. Olivecrona, et al., "Postprandial lipemia, fenofibrate and coronary artery disease," *Atherosclerosis*, vol. 85, no. 2-3, pp. 193–202, 1990.

[16] J. M. Peters, N. Hennuyer, B. Staels, et al., "Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor α-deficient mice," *The Journal of Biological Chemistry*, vol. 272, no. 43, pp. 27307–27312, 1997.

[17] P. Costet, C. Legendre, J. Moré, A. Edgar, P. Galtier, and T. Pineau, "Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis," *The Journal of Biological Chemistry*, vol. 273, no. 45, pp. 29577–29585, 1998.

[18] C. Duval, M. Müller, and S. Kersten, "PPARα and dyslipidemia," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 961–971, 2007.

[19] V. Zoete, A. Grosdidier, and O. Michielin, "Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 915–925, 2007.

[20] M. H. Liu, J. Li, P. Shen, B. Husna, E. S. Tai, and E. L. Yong, "A natural polymorphism in peroxisome proliferator-activated receptor-α hinge region attenuates transcription due to defective release of nuclear receptor corepressor from chromatin," *Molecular Endocrinology*, vol. 22, no. 5, pp. 1078–1092, 2008.

[21] D. Caron-Dorval, P. Paquet, A.-M. Paradis, et al., "Effect of the PPAR-alpha L162V polymorphism on the cardiovascular disease risk factor in response to *n*-3 polyunsaturated fatty acids," *Journal of Nutrigenetics and Nutrigenomics*, vol. 1, no. 4, pp. 205–212, 2008.

[22] E. M. Balk, A. H. Lichtenstein, M. Chung, B. Kupelnick, P. Chew, and J. Lau, "Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review," *Atherosclerosis*, vol. 189, no. 1, pp. 19–30, 2006.

[23] W. S. Harris, "n-3 fatty acids and serum lipoproteins: human studies," *American Journal of Clinical Nutrition*, vol. 65, no. 5, pp. 1645S–1654S, 1997.

[24] S.-H. Wong, E. A. Fisher, and J. B. Marsh, "Effects of eicosapentaenoic and docosahexaenoic acids on apoprotein B mRNA and secretion of very low density lipoprotein in HepG2 cells," *Arteriosclerosis*, vol. 9, no. 6, pp. 836–841, 1989.

[25] Y. Kobatake, K. Kuroda, and H. Jinnouchi, "Differential effects of dietary eicosapentaenoic and docosahexaenoic fatty acids on lowering of triglyceride and cholesterol levels in the serum of rats on hypercholesterolemic diet," *Journal of Nutritional Science and Vitaminology*, vol. 30, no. 4, pp. 357–372, 1984.

[26] N. Willumsen, S. Hexeberg, J. Skorve, M. Lundquist, and R. K. Berge, "Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty acid oxidation in liver of rats," *Journal of Lipid Research*, vol. 34, no. 1, pp. 13–22, 1993.

[27] I. Ikeda, K. Wakamatsu, A. Inayoshi, K. Imaizumi, M. Sugano, and K. Yazawa, "α-linolenic, eicosapentaenoic and docosahexaenoic acids affect lipid metabolism differently in rats," *Journal of Nutrition*, vol. 124, no. 10, pp. 1898–1906, 1994.

[28] I. Ikeda, J.-Y. Cha, T. Yanagita, et al., "Effects of dietary α-linolenic, eicosapentaenoic and docosahexaenoic acids on hepatic lipogenesis and β-oxidation in rats," *Bioscience, Biotechnology and Biochemistry*, vol. 62, no. 4, pp. 675–680, 1998.

[29] G. S. Rambjør, A. I. Wålen, S. L. Windsor, and W. S. Harris, "Eicosapentaenoic acid is primarily responsible for hypotriglyceridemic effect of fish oil in humans," *Lipids*, vol. 31, no. 1, pp. S45–S49, 1996.

[30] S. Grimsgaard, K. H. Bønaa, J.-B. Hansen, and A. Nordøy, "Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids," *American Journal of Clinical Nutrition*, vol. 66, no. 3, pp. 649–659, 1997.

[31] L. M. Sanderson, P. J. de Groot, G. J. E. J. Hooiveld, et al., "Effect of synthetic dietary triglycerides: a novel research paradigm for nutrigenomics," *PLoS ONE*, vol. 3, no. 2, p. e1681, 2008.

[32] H. M. de Vogel-van den Bosch, M. Bünger, P. J. de Groot, H. Bosch-Vermeulen, G. J. E. J. Hooiveld, and M. Müller, "PPARalpha-mediated effects of dietary lipids on intestinal barrier gene expression," *BMC Genomics*, vol. 9, article 231, pp. 1–13, 2008.

Hindawi Publishing Corporation
PPAR Research
Volume 2009, Article ID 193413, 8 pages
doi:10.1155/2009/193413

Research Article

Regulation of Translational Efficiency by Disparate 5′-UTRs of PPARγ Splice Variants

Shawn McClelland, Roopali Shrivastava, and Jheem D. Medh

Department of Chemistry and Biochemistry, California State University Northridge, Northridge, CA 91330-8262, USA

Correspondence should be addressed to Jheem D. Medh, jheem.medh@csun.edu

Received 30 June 2009; Accepted 1 September 2009

Recommended by Mostafa Badr

The PPAR-γ gene encodes for at least 7 unique transcripts due to alternative splicing of five exons in the 5′-untranslated region (UTR). The translated region is encoded by exons 1–6, which are identical in all isoforms. This study investigated the role of the 5′-UTR in regulating the efficiency with which the message is translated to protein. A coupled *in vitro* transcription-translation assay demonstrated that PPAR-γ1, -γ2, and -γ5 are efficiently translated, whereas PPAR-γ4 and -γ7 are poorly translated. An *in vivo* reporter gene assay using each 5′-UTR upstream of the firefly luciferase gene showed that the 5′-UTRs for PPAR-γ1, -γ2, and -γ4 enhanced translation, whereas the 5′-UTRs for PPAR-γ5 and -γ7 inhibited translation. Models of RNA secondary structure, obtained by the mfold software, were used to explain the mechanism of regulation by each 5′-UTR. In general, it was found that the translational efficiency was inversely correlated with the stability of the mRNA secondary structure, the presence of base-pairing in the consensus Kozak sequence, the number of start codons in the 5′-UTR, and the length of the 5′-UTR. A better understanding of posttranscriptional regulation of translation will allow modulation of protein levels without altering transcription.

1. Introduction

Peroxisome proliferators-activated receptors (PPAR) are a family of nuclear receptors associated with cellular differentiation, and with the regulation of carbohydrate and lipid metabolism [1, 2]. PPAR consist of three main subtypes, PPAR-α, PPAR-β and PPAR-γ. Of these, PPAR-γ is the most extensively studied as it is implicated in several pathophysiological processes [3–5]. PPAR-γ are transcription factors that dimerize with the retinoid X receptor (RXR), and the heterodimers bind to specific DNA target sequences called PPAR response elements (PPREs) [6]. Numerous genes implicated in inflammation, cardiovascular disease, diabetes, and obesity are known to have a PPRE [7, 8]. Thus, the influence of PPAR on a cell is manifold and complex.

The PPAR-γ gene is found at chromosome 3p25 in humans [9]. Although transcription derives from only this one gene, several mRNA splice variants have been found [10, 11]. All splice variants consist of exons 1 through 6 consecutively on the 3′ end of the mRNA; these exons code for most of the actual PPAR-γ protein. The 5′ end of the mRNA consists of alternately spliced exons A1, A2, B, C, and D in various combinations to form seven splice variants. In each splice variant the exons at the 5′ end account for little or none of the final translated PPAR-γ protein. A schematic of the splice variants and their PPAR-γ protein start codons (ATG) can be seen in Figure 1.

The biological significance of the existence of multiple PPAR-γ transcript isoforms that encode for identical protein isoforms is not yet clear. The splice variants differ only in the 5′-UTR. It is likely that this region may contribute to posttranscriptional regulation of PPAR-γ protein expression. The 5′ UTR of apolipoprotein B was shown to increase the efficiency of translation in luciferase reporter gene assays and by *in vitro* translation assays [12]. The expression of serum amyloid A2 apolipoprotein was also posttranscriptionally regulated by both its 5′- and 3′-UTRs [13]. The translation of glutamate receptor 2 is inhibited by a polymorphic repeat sequence in its 5′-UTR [14]. Similarly, differences in their 5′-UTRs may influence the translational efficiency of PPAR-γ transcripts.

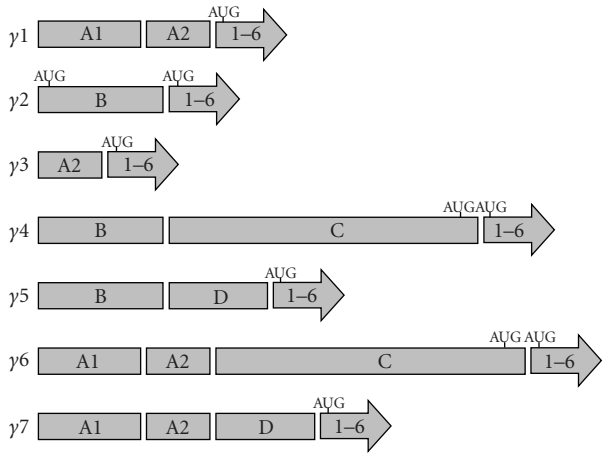


Figure 1: *Structures of* PPARγ *splice variants.* Alignment of exons in seven PPARγ mRNA splice variants is shown with 5′ end on the left and 3′ end on the right. The major translation initiation sites for each isoform are marked (ATG).

There are several mechanisms by which the 5′-UTR may regulate translation. The presence of secondary stem-loop structures or short open-reading frames (ORFs) in the 5′-UTR considerably compromises translation efficiency [15]. Stable stem-and-loop structures in the 5′-UTR have been shown to block the migration of 40 S ribosomes during translation [16]. While moving along the transcript, the 40 S ribosomal subunit scans and evaluates initiation codons sequentially, starting at the 5′-end of the mRNA. The presence of short ORFs in the 5′-UTR allows the initiation complex to remain bound to the RNA even after wasteful translation of the short peptide. Thus, a small ORF greatly reduces but does not eliminate translation of the correct polypeptide [17, 18]. Other factors that affect posttranscriptional regulation of translation include the length of the 5′ UTR, and the sequence context of the initiation codon.

In this study, we have investigated the influence of variable 5′-UTRs on the translation efficiency of PPAR-γ transcripts. All other variables being set equal, PPAR-γ makes for an excellent model to study the translational efficiency due to disparate 5′ UTR, but nearly identical translated regions. As for transcription factors, PPAR-γ may have a significant effect on non-mRNA-sequence elements involved in translation such as eIFs, ribosomes, and phosphorylation. However, the use of *in vitro* translation of specific splice variants excluded such factors from the equation, and any variability in translational efficiency could be attributed to the 5′ UTR. Additionally, the *in vivo* translational efficiency of each of the 5′-UTRs was compared using luciferase reporter gene assays [17, 19]. The experimental data were explained by *in silico* analysis of the whole transcript structures.

As explained above, a primary mechanism for the regulation of translation is the formation of stem-loop secondary structures upstream of the initiation AUG [15]. The formation of mRNA secondary structure can be accurately predicted by computer programs that take into consideration mRNA sequence data, and the free energy change for the formation of various folded structures [18, 20]. The energy minimizing software mfold has been successfully used to investigate various areas of genomics. RNA folding software predicted how mutations in the 5′-UTR of hepatitis C virus RNA altered their stem-loop structure, thermodynamic stability, and binding affinity for ribosomal proteins [21]. The MFOLD software helped to identify a 35-nucleotide unfolded stretch in the 5′-UTR of the human cyclin dependent kinase inhibitor $p27^{Kip1}$, indicating that this region may be the ribosomal recruitment site [22]. With the current efficacy of computer fold modeling, such analysis is a valuable tool in correlating translational efficiency of different PPAR-γ transcript isoforms to variations in mRNA secondary structure.

2. Materials and Methods

2.1. Preparation of Constructs with a T7 Polymerase Promoter. A T7 promoter was an added upstream of full-length DNA splice variants for PPARγ1, γ2, γ4, γ5, and γ7. This was accomplished by the PCR technique using previously cloned full-length genes, for each isoform, and specific primer sets. The sense primers were engineered to contain the T7 promoter sequence. For γ1 and γ7 the sense primer T7A1 (5′ TAA TAC GAC TCA CTA TAG GGC CTT TAC CTC TGC TGG TGA C 3′) was used. For the remaining splice variants the sense primer T7B (5′ TAA TAC GAC TCA CTA TAG GGA GCA AAC CCC TAT TCC ATG C 3′) was used. The same anti-sense primer (PPARγ-antisense) was used for all splice variants (5′ CTA AAA CCG TTT CTT TTT AAA ATG C 3′) since they have identical 3′ ends. PCR was run using a 60°C annealing temperature and 40 cycles. A blank sample was created by using distilled water instead of a DNA template, the sense T7A1, and the antisense primers.

The resulting products were resolved on a 1% agarose gel and displayed the expected sizes for the templates of interest (data not shown). The bands corresponding to each desired full-length splice variant with T7 promoter were excised (as well as the empty area where the band would be in the case of the blank) and DNA was extracted using a Geneclean II kit (QBiogene Irvine, CA). The amount of DNA was quantitated and the samples were stored in the −20°C freezer for future use in a linked *in vitro* transcription-translation reaction.

2.2. Linked In Vitro Transcription-Translation. Linked *in vitro* transcription-translation was performed using a Proteinscript II T7 kit from Ambion (Applied Biosystems, Foster City, CA). The kit allows coupled *in vitro* transcription and translation from a DNA template containing a T7 promoter upstream of the DNA to be transcribed. Equal amounts of gel-extracted DNA template (0.5 μg of DNA for each template) for each splice variant were used for the reaction. The blank sample was used as a negative control and plasmid pTRI-Xef, provided with the kit, was used as a positive control. In one experiment, the pTRI-Xef template was mixed with DNA template for each splice variant to determine whether the DNA or mRNA of the specific

splice variant inhibited translational efficiency of unrelated genes. The templates (6 μL) were mixed with 2 μL of 5X transcription mix and 2 μL of T7 polymerase. The reaction was allowed to incubate at 30°C for 60 minutes and then placed on ice. At this time, the DNA templates had been transcribed into mRNA which could be run immediately in the *in vitro* translation step, or could be stored at −20°C for future use.

In vitro translation was carried out by first making a master mix of 24 μL per each sample using 2 μL nuclease free water, 1.25 μL 20X translation mix, 1.25 μL unlabeled methionine (500 μM), 2 μL [^{35}S]-methionine, and 17.5 μL retic lysate. To 24 μL of the master mix, 1 μL of the previously transcribed mRNA samples were added. The tubes were then gently mixed and incubated at 30°C for 60 minutes, after which they were immediately transferred to ice to stop the reaction. The products were now proteins with the incorporated radiolabeled methionine.

2.3. Analysis of In Vitro Translated Proteins. Analysis of the radiolabeled proteins was done by two different methods. First, a portion of the sample was precipitated using trichloroacetic acid (TCA). Briefly, 5 μL of the translation product was mixed with 500 μL of decolorizing solution (1 M NaOH, 1.5% H_2O_2, 1 mM L-Methionine) and 250 μL of distilled water. The tubes were incubated for 10 minutes at 30°C followed by the addition of 1 mL of 25% TCA to precipitate the proteins. The pellets were dissolved in water and the amount of radioactivity was measured using a Beckman LS 6500 liquid scintillation counter. In another approach, the *in vitro* translation products were resolved by SDS-PAGE on a 12% acrylamide gel. The gel was fixed in a solution of 50% methanol and 7% acetic acid, washed, and soaked in a solution of 1 M sodium salicylate with 50% glycerol to enhance fluorographic detection of radiolabeled proteins. The gel was dried under vacuum at 80°C, and exposed to autoradiography film (Kodak BioMax MR) for one to four days at −80°C The films were developed using a Kodak RP X-OMAT Processor, scanned, and subjected to quantitative analysis of band intensity.

2.4. ImageJ Analysis. The relative abundance of *in vitro* translated proteins was determined by a densitometric analysis of the bands detected upon by autoradiography of the dried gels. An imaging software, ImageJ, available at the National Institutes of Health (NIH) website was used. Each band's intensity was quantitated by measuring the integrated density of a box made around each band and subtracting out the integrated density of the same sized box of the image's background. The band intensity of the darkest band was set to the value of one hundred and the intensities of other bands were assigned an adjusted relative value.

2.5. Dual Luciferase Reporter Gene Assay. The translational efficiency of each 5′-UTR was also measured through reporter gene constructs using the dual luciferase reporter (DLR) assay kit from Promega [17]. This method involves the cotransfection of chimeric firefly luciferase reporter gene constructs with a control construct expressing the *Renilla* luciferase gene. This allows for correction for any variation in transfection efficiency. The protocol described in the DLR assay kit was followed. For construction of the reporter gene vectors, the 5′ UTR of each of the different PPAR-γ transcript isoforms was PCR amplified using plasmids containing the corresponding full-length PPAR-γ DNA as templates, and specific primer pairs for each 5′-UTR, as shown in Table 1. The primers were designed with *Mlu I* and *Bgl II* restriction sites, to facilitate cloning into the pGL3-promoter luciferase reporter vector (Promega). After amplification, the PCR products were digested with Mlu I and Bgl II, gel purified, and cloned into the pGL3-promoter vector upstream of the firefly luciferase gene. Appropriate insertion of the 5′-UTR fragments was confirmed by sequencing from both ends using sequencing primers provided with the vector. The chimeric reporter gene (firefly luciferase) constructs were mixed with an expression vector for *Renilla* luciferase gene in a 9 : 1 mass ratio, and the two plasmids were transiently cotransfected into rat muscle cells (L6 cells) using Lipofectamine 2000. After 4 days, cell lysates were prepared and analyzed for levels of both firefly and *Renilla* luciferase activities using the DLR assay kit (Promega).

2.6. Statistical RNA Folding. The mfold server located at http://mfold.bioinfo.rpi.edu/ was used to model how the different splice variant mRNAs would fold [20]. Full sequences for each splice variant [11], obtained from the National Center for Biotechnology Information (NCBI), were analyzed using the Rensselaer Polytechnic Institute's web server running the mfold program. The mRNA sequences were simulated as though they were at 37°C in 1 M NaCl. Although physiological conditions typically maintain an ionic strength that is lower than 1 Molar, the conditions used in the simulation, 1 M NaCl is the current standard used in fold modeling. This standard is used in order to better compare modeling results between distinct experiments and simulations. Free energies and secondary structures for each splice variant were evaluated to discover possible correlations between experimental observations and stability and folding patterns of secondary structures. Other mRNA folding programs were also used to simulate mRNA secondary structures. In each case, nearly identical results were obtained, giving support to our mfold results.

3. Results and Discussion

3.1. In Vitro Transcription-Translation. Transfection studies with *in vivo* overexpression of PPARγ splice variants suggested that they were translated with different efficiencies (data not shown). We, therefore, used an *in vitro* linked transcription-translation assay to examine the translational efficiencies of PPARγ splice variants. Using this approach, we were able to examine the translational efficiency of each splice variant without regard to possible feedback inhibition or competition with other splice variants.

Figure 2(a) shows results of a linked transcription-translation assay. Panel A is a representative autoradiogram of ^{35}S-labeled proteins resolved by SDS-PAGE. It is evident that PPARγ4 and PPARγ7 are translated considerably

Table 1: Primers for amplification of different PPAR-γ 5′-UTRs.

5′ UTR of	Sense primer	Antisense primer	Size (bp)
PPAR-γ1	ATCACGCGTCCTTTACCTCTGCTGGTGACA	CGGAGATCTTGTTAAAGGCTGACTCTTGTT	177
PPAR-γ2	GTAACGCGTAGCAAACCCCTATTCCATGCT	CGGAGATCTCTTGTGATATGTTTGCAGACA	142
PPAR-γ4	GTAACGCGTAGCAAACCCCTATTCCATGCT	CGGAGATCTCTGAAAAGCCTTTCATAGGTC	405
PPAR-γ5	GTAACGCGTAGCAAACCCCTATTCCATGCT	CGAAGATCTTAATCCCAGCACTTTGGGAGG	221
PPAR-γ7	ATCACGCGTCCTTTACCTCTGCTGGTGACA	CGAAGATCTTAATCCCAGCACTTTGGGAGG	256

less than PPARγ2 and PPARγ1. The translation of PPAR-γ5 was just slightly less than that of PPAR-γ1. The PPARγ2 splice variant has two working start codons and translates into both the PPARγ1 protein isoform and the PPARγ2 protein isoform. The other splice variants translate to mainly the γ1 protein isoform. The PPARγ4 transcript also has an additional initiation codon that would add 8 amino acids to the aminoterminus of the protein; however, such a protein isoform was not seen. The intensities for each band were quantitated using ImageJ (Figure 2(b)). The values are represented as a percent of the most intense band (resulting from the γ2 splice variant template) which was set to a value of 100.

The products of the linked *in vitro* transcription-translation reaction were subjected to a TCA protein precipitation, and the radioactivity in the precipitated proteins was measured using a liquid scintillation counter (Figure 2(b)). Counts were adjusted such that the PPARγ2 count equaled 100. The results corresponded closely to the SDS-PAGE-autoradiography values. Figure 2(b) shows data representative of two replicate independent experiments where the splice variants for PPAR-γ1 and PPAR-γ2 were consistently more efficiently translated than those for PPAR-γ4 and -γ7.

Our data indicated that the different 5′-UTRs of PPARγ had a regulatory effect on the ability of the transcript to be translated. It was possible that the relatively lengthy 5′-UTRs of PPARγ4 (389 bases) and -γ7 (240 bases) could sequester the translation machinery of the cell and impose a broad repression of translation of all cellular transcripts. To examine this possibility, the DNA templates of each PPARγ splice variant were mixed with a reference DNA template, pTRI-Xef, that was supplied with the Proteinscript II kit (Ambion). Results of linked *in vitro* transcription-translation using the mixed templates, as well as of standard pTRI-Xef alone, are shown in Figure 2(c). After SDS-PAGE, the intensities of all samples were quantified and are shown below the corresponding bands, but they fell within 5% of each other. This experiment shows that templates or transcripts for any of the PPAR-γ splice variants do not slow down translation of an unrelated protein in an *in vitro* assay.

3.2. In Vivo Reporter Gene Assays. Next, we studied regulation of *in vivo* translation by each of the PPAR-γ 5′-UTRs using a luciferase reporter gene assay. When equal amounts of 5′-UTR-firefly luciferase gene constructs were transfected into cultured L6 cells (an easily transfected rat muscle cell line), and cell lysates corrected for equal protein mass were used, the expression of luciferase activity was significantly enhanced compared to control (pGL3 vector without any

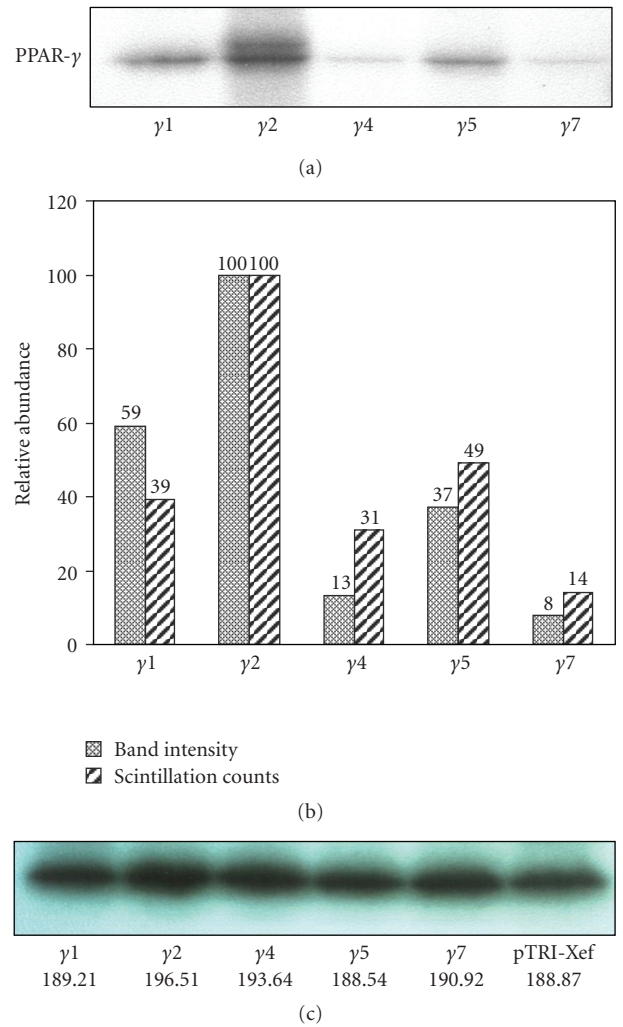


Figure 2: *In vitro Transcription-Translation.* Templates for the full-length PPAR-γ1, γ2, γ4, γ5, and γ7 splice variants were used to perform coupled *in vitro* transcription and translation reactions as described in "Methods". Products were separated using SDS-PAGE. (a). The gel was dried and exposed to x-ray film to visualize *in vitro* labeled protein products. (b). *(dotted bars)* Band intensities were quantitated and the most optically dense band was set to 100. (b). *(hatched bars)* After the coupled *in vitro* transcription-translation reaction, labeled proteins were precipitated using TCA and the radioactivity in the precipitate was measured using a liquid scintillation counter. The highest counts were set to 100. (c). Templates for the γ1, γ2, γ4, γ5, and γ7 splice variants were mixed with a pTRI-Xef template and *in vitro* transcription and translation reactions were performed on each mixture. Bands were resolved by SDS-PAGE. The bands for pTRI-Xef are shown with the band intensities reported below each band.

Table 2: Comparison of structural elements of 5′-UTRs of PPAR-γ transcript isoforms.

Splice variant	No. of start codons	No. of start codons in 5′-UTR	Length of 5′-UTR (bases)	Strength of Kozak sequence (No. of matching bases)	No. of Kozak sequence bases bound in secondary structure	$\triangle G^\circ$ from energy dot plots kCal/mol
$\gamma 1$	37	0	141	7	2	−516
$\gamma 2$	39	2	106	4, 7	3, 3	−507
$\gamma 4$	49	12	369	4, 7	3, 3	−590
$\gamma 5$	40	3	190	7	2	−543
$\gamma 7$	38	1	225	7	7	−547

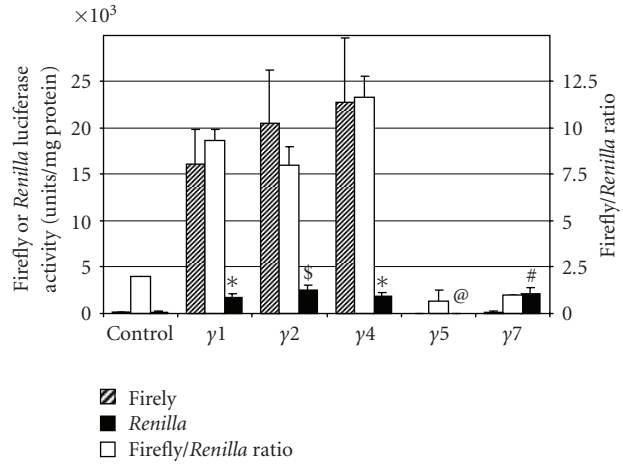


Figure 3: *In vivo reporter gene assays.* Firefly luciferase reporter gene constructs were designed to contain individual 5′-UTRs upstream of the luciferase reporter gene in the pGL3 vector (Promega). Rat L6 cells were transfected with either the host pGL3 vector (control) or the vector containing a specific PPAR-γ 5′-UTR. Cells were cotransfected with a plasmid expressing the *Renilla* luciferase enzyme. After 4 days, cell lysates were prepared and analyzed for firefly (hatched bars) and *Renilla* (solid bars) luciferase activities. The open bars show the ratio of firefly to *Renilla* luciferase ($n = 3$, P values relative to control are $* < .005$, $\# < .05$, $\$ < .01$, $@ < 0.5$).

insert upstream of the luciferase gene) when the 5′-UTRs for PPAR-γ1, -γ2, and γ4 were inserted upstream of the firefly luciferase reporter gene (Figure 3, hatched bars). The firefly luciferase activity was not altered relative to control when the 5′-UTR for PAPR-γ7 was inserted upstream of the reporter gene, whereas it was repressed and was undetectable when the 5′-UTR of PPAR-γ5 was inserted in the vector. The activity for the cotransfected *Renilla* luciferase was much lower than the firefly luciferase (Figure 3, solid bars). This was consistent with the transfection ratio of 9 : 1 for firefly: *Renilla* luciferase plasmids. Control experiments with purified firefly luciferase and *Renilla* luciferase enzymes confirmed that mixing the two enzymes did not interfere with the quantitative measurement of individual enzyme activities (data not shown). The *Renilla* luciferase activity was several fold greater than control in cells cotransfected with firefly luciferase constructs containing the 5′-UTRs for PPAR-γ1, PPAR-γ2, PPAR-γ4, and PPAR-γ7, but it was undetectable in cells cotransfected with PPAR-γ5 5′-UTR-luciferase contructs. Since the *Renilla* luciferase served as a transfection control, results are also expressed as a ratio of the firefly luciferase to the *Renilla* luciferase activities (Figure 3, open bars, $n = 3$, P values are shown), as is customary for the DLR assay [17]. The ratio of the two enzymes was the highest for cells transfected with the 5′-UTRs for PPAR-γ4, PPAR-γ1, and PPAR-γ2. The results for the *in vivo* DLR assay suggest that the presence of these three 5′-UTRs enhance translation, whereas the 5′-UTRs for PPAR-γ5 and PPAR-γ7 repress translation of the firefly luciferase gene, compared to control. Interestingly, even though the expression of *Renilla* luciferase activity is used as a control for transfection efficiency, and is expected to vary randomly, its expression showed the exact pattern in multiple experiments, with the level being relatively high (compared to control) when cotransfected with reporter gene constructs containing 5′-UTRs of PPAR-γ1, -γ2, -γ4, or -γ7. The presence of the PPAR-γ5 5′-UTR always failed to stimulate *Renilla* luciferase activity. Since the *Renilla* luciferase gene is itself not driven by any variable cis-acting upstream elements in the different transfections, our results suggest that perhaps the 5′-UTRs inserted in the cotransfected reporter gene constructs may be influencing the translation of *Renilla* luciferase in a transacting manner. This is contrary to the absence of any trans regulation in the *in vitro* assay (Figure 2(c)); however, the presence of cellular elements in the *in vivo* DLR assay may contribute factors that may bind to and regulate the activities of both the *Renilla* and firefly luciferase enzymes. It may be interesting to investigate whether this trans-regulation is different in different cell types.

3.3. RNA Fold Modeling. In order to explain differences in translational efficiency for each splice variant, we examined differences in the primary and secondary structures of each transcript variant. Recent advances in computational modeling of DNA and RNA have made such an investigation a viable approach.

Using the mfold RNA-folding software, each splice variant's full mRNA sequence was computationally folded. The mfold software reports several folded structures along with the free energy change of folding. In order to predict the average stability of secondary structures, mfold also calculates the probability of interactions between any two bases in the input sequence. It prepares an energy dot plot, where each dot represents a possible base pair formation and a chain of dots represents possible helical structures. Such

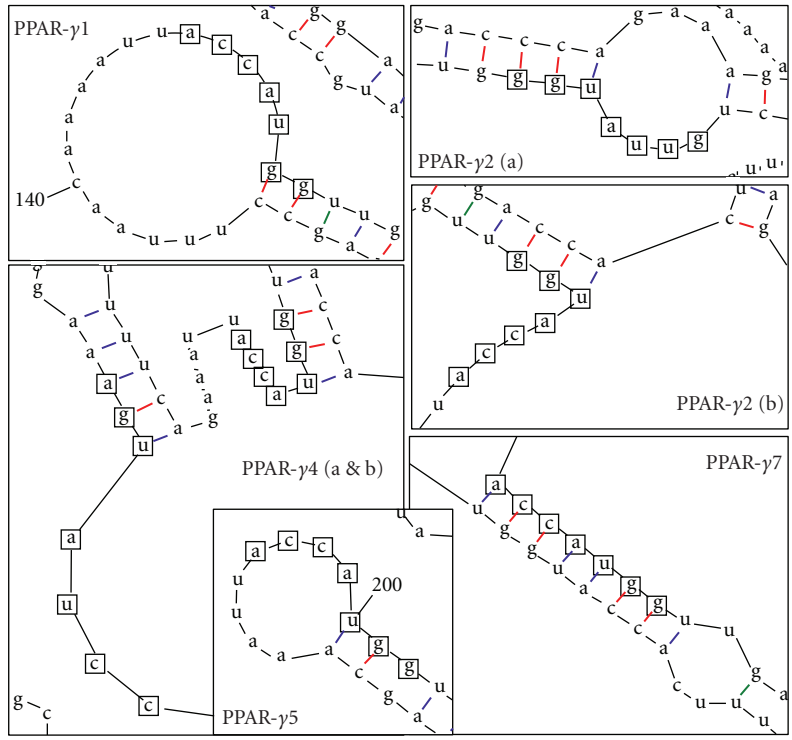


Figure 4: *Magnified RNA folding Models.* Full-length PPAR-γ splice variants were folded using the mfold software. The start codon regions for each isoform are magnified here to show the canonical start codon region (accAUGg) and the number of bases in that region that are bound.

energy plots were much denser for PPAR-γ4, PPAR-γ5, and PPAR-γ7 compared to those for PPAR-γ1 and PPAR-γ2 (not shown) indicating the lower stability of secondary structures for PPAR-γ1 and PPAR-γ2. Table 2 shows the optimum free energy change for secondary structure formation computed by such energy dot plots.

The most stable structures with the lowest free energy change were used to magnify the start codon regions for each of the splice variants (Figure 4). Closer inspection of secondary structures reveals possible indicators of each splice variant's translational efficiency. Some factors that are known to reduce translation efficiency are longer 5′-UTRs with multiple start codons that may result in false starts or short ORF segments that lead to nonsense products [15]. Both PPARγ1 and PPARγ2 splice variants translated efficiently due to the fact that their secondary structures are the least stable, they have the shortest 5′-UTRs, and the fewest start codons in their 5'-UTRs (Table 2). The PPARγ7 splice variant also has only 1 start codon in its 5′-UTR, but it is very poorly translated. To explain this, we examined the sequence around the start codon more closely. The ribosome of the translation initiation complex recognizes a Kozak consensus sequence at the translation start site [23]. In Figure 4, this region accAUGg is highlighted and the number of consensus bases that are bound in the secondary structure is reported in Table 2. For PPARγ7, all seven bases of the start motif completely match the Kozak sequence, and each base in the region is bound in a secondary structure. This may be the reason why this splice variant is translated very inefficiently during the *in vitro* and *in vivo* translation experiments. Similarly, the upstream alternative start codons in PPAR-γ2 and PPAR-γ4 (indicated as PPAR-γ2 b and PPAR-γ4 b in Figure 4) have a weaker consensus motif and this may be the reason why the larger size proteins were not efficiently translated in Figure 2. The *in vitro* experiment (Figure 2) is also consistent with the predicted translational efficiency for PPAR-γ4 and PPAR-γ5. PPAR-γ4 is most inefficiently translated due to the finding that its secondary structure is most stable (lowest $\triangle G^\circ$ values), and it has the longest 5′-UTR with 12 putative start codons. On the other hand, translation of PPAR-γ5 is intermediate, since none of the inhibitory factors are extreme. The *in vivo* experiments with luciferase reporter gene assays indicate that translational efficiency of PPAR-γ4 is very efficient. This could be due to the presence of cellular factors that may bind to the 5′-UTR and promote translation. Certain nucleotide sequences in the 5′-UTR may form secondary structures that can function as internal ribosome entry sites (IRES) [24]. These structures require additional cellular proteins called IRES trans-acting factors (ITAFs) to promote translation [25]. Since ITAFs would be present in the *in vivo* model but absent in the *in vitro* translation, it explains why the PPAR-γ4 5′-UTR drives translation *in vivo* but not *in vitro*.

4. Conclusion

The reason for the existence of splice variants is a source of much speculation. The foremost theory is that it provides

greater flexibility and diversity of protein expression without the need for more DNA. However, in the case of PPARγ, the splice variants seem to play a different role. As opposed to producing varying proteins, they produce essentially the same protein. Instead, the PPARγ splice variants appear to regulate protein expression. While other mechanisms of regulation may also contribute, a primary mechanism appears to be the differing translational efficiencies of the many splice variants.

In an attempt to explicate what is causing the differences in translational efficiency, we turned to RNA secondary structure modeling. Previous studies have attributed 5′-UTR length and number of start codons as key factors in changing translational efficiencies of mRNA strands [26, 27]. However, these factors alone did not seem to completely explain translation of the PPARγ splice variants. Length and number of start codons are both attributes related to the primary sequence of the RNA. Looking at secondary structure gave a better insight and understanding of the role of 5′-UTRs in regulation of translation. As research progresses in the area of mRNA secondary structure and its interactions, we will be even better at predicting translational efficiencies. For instance, as the ability to identify riboswitch sequences increases, we would be able to determine whether different riboswitches present in different splice variants influence translational efficiency.

As we better understand the regulatory functions of each PPAR-γ splice variant, it will become possible to modulate PPAR-γ protein expression and therefore its end cellular effect. This will lead to better treatments and management of countless diseases that PPAR-γ is implicated in.

5. Acknowledgments

This work was supported by a National Institutes of Health MBRS-SCORE Award (S06 GM8680) and a National Institutes of Health AREA Grant (R15 HL083946). We thank Kumuda Saraff for helpful discussions and assistance at the bench.

References

[1] M.-C. Cho, K. Lee, S.-G. Paik, and D.-Y. Yoon, "Peroxisome proliferators-activated receptor (PPAR) modulators and metabolic disorders," *PPAR Research*, vol. 2008, Article ID 679137, 14 pages, 2008.

[2] T. M. Willson, P. J. Brown, D. D. Sternbach, and B. R. Henke, "The PPARs: from orphan receptors to drug discovery," *Journal of Medicinal Chemistry*, vol. 43, no. 4, pp. 527–550, 2000.

[3] L. Széles, D. Töröcsik, and L. Nagy, "PPARγ in immunity and inflammation: cell types and diseases," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 1014–1030, 2007.

[4] C. K. Glass, "Potential roles of the peroxisome proliferator-activated receptor-γ in macrophage biology and atherosclerosis," *Journal of Endocrinology*, vol. 169, no. 3, pp. 461–464, 2001.

[5] W. He, "PPAR$\gamma 2^{\text{Pro12Ala}}$ polymorphism and human health," *PPAR Research*, vol. 2009, Article ID 849538, 15 pages, 2009.

[6] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[7] D. G. Lemay and D. H. Hwang, "Genome-wide identification of peroxisome proliferator response elements using integrated computational genomics," *Journal of Lipid Research*, vol. 47, no. 7, pp. 1583–1587, 2006.

[8] M. S. Hamza, S. Pott, V. B. Vega, et al., "De-novo identification of PPARγ/RXR binding sites and direct targets during adipogenesis," *PLoS ONE*, vol. 4, no. 3, article e4907, 116 pages, 2009.

[9] D. C. Bittel, N. Kibiryeva, M. Dasouki, J. H. M. Knoll, and M. G. Butler, "A 9-year-old male with a duplication of chromosome 3p25.3p26.2: clinical report and gene expression analysis," *American Journal of Medical Genetics A*, vol. 140, no. 6, pp. 573–579, 2006.

[10] Y. Chen, A. R. Jimenez, and J. D. Medh, "Identification and regulation of novel PPAR-γ splice variants in human THP-1 macrophages," *Biochimica et Biophysica Acta*, vol. 1759, no. 1-2, pp. 32–43, 2006.

[11] J. Zhou, K. M. Wilson, and J. D. Medh, "Genetic analysis of four novel peroxisome proliferator activated receptor-γ splice variants in monkey macrophages," *Biochemical and Biophysical Research Communications*, vol. 292, no. 1, pp. 274–283, 2002.

[12] L. Pontrelli, K. G. Sidiropoulos, and K. Adeli, "Translational control of apolipoprotein B mRNA: regulation via cis elements in the 5′ and 3′ untranslated regions," *Biochemistry*, vol. 43, no. 21, pp. 6734–6744, 2004.

[13] D. B. Longley, D. M. Steel, and A. S. Whitehead, "Post-transcriptional regulation of acute phase serum amyloid A2 expression by the 5′- and 3′-untranslated-regions of its mRNA," *Journal of Immunology*, vol. 163, no. 8, pp. 4537–4545, 1999.

[14] S. J. Myers, Y. Huang, T. Genetta, and R. Dingledine, "Inhibition of Glutamate Receptor 2 Translation by a Polymorphic Repeat Sequence in the 5′-ntranslated Leaders," *Journal of Neuroscience*, vol. 24, no. 14, pp. 3489–3499, 2004.

[15] M. Kozak, "Structural features in eukaryotic mRNAs that modulate the initiation of translation," *The Journal of Biological Chemistry*, vol. 266, no. 30, pp. 19867–19870, 1991.

[16] B. C. Morrish and M. G. Rumsby, "The 5′ UTR of protein kinase C ε confers translational regulation in vitro and in vivo," *Biochemical and Biophysical Research Communications*, vol. 283, no. 5, pp. 1091–1098, 2001.

[17] B. A. Sherf, S. L. Navarro, R. R. Hannah, and K. V. Wood, "Dual-luciferase reporter assay: an advanced co-reporter technology integrating firefly and *Renilla* luciferase assays," *Promega Notes*, vol. 57, pp. 2–9, 1996.

[18] D. H. Mathews, J. Sabina, M. Zuker, and D. H. Turner, "Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure," *Journal of Molecular Biology*, vol. 288, no. 5, pp. 911–940, 1999.

[19] D. J. Groskreutz, B. A. Sherf, K. V. Wood, and E. T. Schenborn, "Increased expression and convenience with the new pGL3 luciferase reporter vectors," *Promega Notes*, vol. 50, pp. 2–8, 1995.

[20] M. Zuker, "Mfold web server for nucleic acid folding and hybridization prediction," *Nucleic Acids Research*, vol. 31, no. 13, pp. 3406–3415, 2003.

[21] M. K. El Awady, H. A. Azzazy, A. M. Fahmy, et al., "Positional effect of mutations in 5′UTR of hepatitis C virus 4a on patients' response to therapy," *World Journal of Gastroenterology*, vol. 15, no. 12, pp. 1480–1486, 2009.

[22] J. Coleman and W. K. Miskimins, "Structure and activity of the internal ribosome entry site within the human p27Kip1 5′-untranslated region," *RNA Biology*, vol. 6, no. 1, pp. 84–89, 2009.

[23] M. Kozak, "An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs," *Nucleic Acids Research*, vol. 15, no. 20, pp. 8125–8148, 1987.

[24] A. A. Komar and M. Hatzoglou, "Internal ribosome entry sites in cellular mRNAs: mystery of their existence," *The Journal of Biological Chemistry*, vol. 280, no. 25, pp. 23425–23428, 2005.

[25] C. U. T. Hellen and P. Sarnow, "Internal ribosome entry sites in eukaryotic mRNA molecules," *Genes and Development*, vol. 15, no. 13, pp. 1593–1612, 2001.

[26] S. Singh, S. C. Bevan, K. Patil, D. C. Newton, and P. A. Marsden, "Extensive variation in the 5′-UTR of Dicer mRNAs influences translational efficiency," *Biochemical and Biophysical Research Communications*, vol. 335, no. 3, pp. 643–650, 2005.

[27] S. A. Sedman, G. W. Gelembiuk, and J. E. Mertz, "Translation initiation at a downstream AUG occurs with increased efficiency when the upstream AUG is located very close to the 5′ cap," *The Journal of Virology*, vol. 64, no. 1, pp. 453–457, 1990.