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Combined effects of PPAR γ_2 P12A and PPAR α L162V polymorphisms on glucose and insulin homeostasis: the Québec Family Study

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Abstract Peroxisome proliferator-activated receptors γ_2 and α are nuclear factors known to be important regulators of lipid and glucose metabolism. Two polymorphisms, namely PPAR γ_2 P12A and PPAR α L162V, were investigated for their individual and interaction effects on glucose and insulin homeostasis. Genotypes were determined in 663 nondiabetic adults participating in the Québec Family Study and who underwent an oral glucose tolerance test (OGTT). The insulin and C-peptide areas under the curve (AUC) following the OGTT were higher in subjects carrying the PPAR α V162 allele compared to homozygous for the L162 allele. When subjects were grouped according to both polymorphisms, higher levels of insulin and C-peptide during the OGTT were observed for those carrying the PPAR α V162 allele except when they carry at the same time the PPAR γ_2 A12 allele. Thus, the PPAR γ_2 A12 allele seems protective against the deleterious effect of the PPAR α V162 allele. Furthermore, a significant gene-gene inter-

action was observed for the acute (0–30 min) ($p < 0.001$) and the total ($p = 0.05$) C-peptide AUC following the OGTT. These results provide evidence of a gene-gene interaction in the regulation of plasma glucose-insulin homeostasis, and emphasize that these interactions need to be taken into account when dissecting the genetic etiology of complex disorders.

Keywords Peroxisome proliferator-activated receptors · OGTT · Epistasis · Type 2 diabetes · C-peptide · Insulin homeostasis · PPAR γ_2 P12A mutation · PPAR α L162V mutation

Introduction

Understanding the genetic etiology of type 2 diabetes is recognized as an urgent priority (King et al. 1998). The complexity of such a task is related to the multifactorial aspect of the disease. Considerable efforts have been devoted during the past few years to the identification of genes contributing to type 2 diabetes and related phenotypes. However, despite the evidence that there is a strong genetic component to the disease, only few “diabetogenes” have been identified (Busch and Hegele 2001; Newman et al. 1987). Genetic heterogeneity, variable penetrance, gene-gene (epistasis) and gene-environment interactions are among the factors that contribute to the difficulty of identifying the relevant genes.

Epistasis in type 2 diabetes and related phenotypes is likely to occur for genes encoding proteins involved in the regulation of lipid and glucose metabolism. In this regard, genes encoding peroxisome proliferator-activated receptors (PPARs) are relevant candidates. PPARs are members of the superfamily of nuclear hormone receptors (Mangelsdorf et al. 1995). They heterodimerize with the retinoid X receptor and bind to direct repeats within the promoter region of many genes to regulate their transcription (Berger and Moller 2002).

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Three subtypes have been identified, namely PPAR α , γ , and δ . PPARs were first discovered as mediators of peroxisome proliferators (Issemann and Green 1990), but are now considered as key messengers responsible for the translation of nutritional and metabolic stimuli into changes in gene expression (Schoonjans et al. 1997). In addition, PPAR α and γ mediate the effect of fibrates (Staels et al. 1998) and thiazolidinediones (Hauner 2002), respectively, two classes of drugs recognized to regulate lipid and glucose metabolism.

Two common polymorphisms affecting the amino acid sequence of the PPAR α and PPAR γ_2 gene have been identified—PPAR α L162V (Sapone et al. 2000) and PPAR γ P12A (Yen et al. 1997). Three independent studies reported no significant difference in the V162 allele frequency between subjects with and without type 2 diabetes, thus suggesting that the L162V polymorphism in the PPAR α gene does not play a major role in the development of type 2 diabetes (Evans et al. 2001; Lacquemant et al. 2000; Vohl et al. 2000). On the other hand, there is controversy concerning the effect of PPAR γ_2 P12A polymorphism on type 2 diabetes and intermediate phenotypes (Altshuler et al. 2000; Hegele et al. 2000; Mancini et al. 1999). The conflicting results may potentially indicate that the effect of the polymorphism is modulated by environmental and/or other genetic factors.

Recently it has been shown that the effect of PPAR γ_2 P12A polymorphism on insulin sensitivity and insulin levels is dependent on the background of the Gly972Arg polymorphism in the insulin receptor substrate-1 (IRS-1) gene (Stumvoll et al. 2002c) and on the background of the Trp64Arg β_3 -adrenergic receptor gene (Hsueh et al. 2001), respectively. Such examples reinforce the idea that allelic effects at some loci may be attenuated or amplified in the presence of variants at other loci. These interactions may explain the conflicting results observed in some association studies and highlight the importance of investigating gene-gene effects. In addition, incorporating epistasis in analytical models is necessary and important when searching for genes involved in complex traits. The objective of the present study was therefore to study the independent effect of PPAR α L162V and PPAR γ_2 P12A variants as well as their interactive impact on indices of plasma glucose and insulin homeostasis in 663 subjects enrolled in the Québec Family Study (QFS).

Materials and methods

Subjects

The QFS is composed of French Canadian families living in and around the Québec City area, representing a mixture of random sampling and ascertainment through obese (BMI > 32 kg/m²) probands (Bouchard 1996). Only adults aged above 18 years and nondiabetics were included in the present study. A total of 663 subjects (291 men and 372 women) in 241 nuclear families were genotyped for the PPAR γ_2 P12A and the PPAR α L162V poly-

morphisms (age 42.2 ± 17.0 years; BMI: 26.8 ± 6.9 kg/m²). The Medical Ethics Committee of Laval University approved the protocol, and a written consent was obtained from all the subjects.

Glucose, insulin, and C-peptide measurements

Fasting blood samples were collected, and plasma glucose, insulin, and C-peptide levels were measured by standard procedures as previously described (Desbuquois and Aurbach 1971; Heding 1975; Richterich and Dauwalder 1971). Subjects also underwent a 75-g oral glucose tolerance test (OGTT) after an overnight fast. Blood glucose, insulin, and C-peptide levels were measured at -15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min after the glucose load. OGTT areas under the curve (AUC) were calculated using the trapezoid method. The area over the first 30 min defined the acute response, whereas the complete area (0–180 min) was the total response.

DNA analysis

The PPAR γ_2 P12A variant is caused by a C → G substitution at nucleotide 34 producing a BstU-I restriction site. Genotypes were obtained by digestion of PCR products as described in details elsewhere (Yen et al. 1997). The PPAR α L162V polymorphism is caused by a C → G substitution at nucleotide 484 in exon 5 and does not alter any restriction site. A mismatch PCR method previously described was therefore used to genotype individuals of the QFS cohort (Vohl et al. 2000).

Statistical analysis

Besides age and glycemia at 30, 45, and 60 min, all variables under study were abnormally distributed. These variables were normally distributed after a log₁₀ transformation. Antilogarithms of the transformed means were used to obtain geometric means. For statistical comparisons, heterozygous and homozygous carriers of the polymorphic allele were grouped together for both genes and are referred to as PPAR γ_2 X/Ala and PPAR α X/Val. The independent effect of both polymorphisms was tested by comparing the mean phenotype values between carriers and noncarriers using the MIXED procedure implemented in SAS (version 8.2), which takes the nonindependence of family members into account. This procedure is used when the experimental units (in this case the subjects) can be grouped into clusters (family) and the data from a common cluster are possibly correlated.

For all metabolic parameters, age, gender, and logBMI was included in the model. The interaction between the two polymorphisms was tested using two different statistical approaches: First, we constructed a dummy variable that divided the subjects into four genotype combinations, depending upon whether subjects had a variant in neither of the two genes (PPAR γ_2 Pro/Pro + PPAR α Leu/Leu, $n = 456$), in PPAR α only (PPAR γ_2 Pro/Pro + PPAR α X/Val, $n = 75$), in PPAR γ_2 only (PPAR γ_2 X/Ala + PPAR α Leu/Leu, $n = 107$), or in both genes (PPAR γ_2 X/Ala + PPAR α X/Val, $n = 25$). Differences between the four groups were assessed using the MIXED procedure. Secondly, the effects of the PPAR γ_2 P12A, PPAR α L162V and their interaction were quantified in the MIXED model by contrasting the dummy variable for the two main effects (PPAR γ_2 P12A and PPAR α L162V) and the interaction.

Results

The independent effect of each polymorphism on glucose-related and insulin-related variables is presented

in Table 1. In the fasting state, neither polymorphism (P12A and L162V) was associated with glucose, insulin, and C-peptide levels. However, carriers of the PPAR α V162 allele had higher total insulin AUC following the oral glucose load than noncarriers. There was also a trend toward higher acute insulin and C-peptide AUC as well as higher total C-peptide AUC among carriers of the V162 allele.

In order to evaluate the interaction between P12A and L162V polymorphisms, subjects were divided into four genotype groups based on the presence or absence of the two variants. No difference was observed between the four genotype groups for the fasting glucose, insulin, and C-peptide levels (not shown). Fig. 1 illustrates glucose, insulin, and C-peptide responses to the OGTT for each genotype group. Glucose and insulin levels during the OGTT as well as the glucose and insulin acute and total AUC were not different between the four groups (Figs. 1A and B). However, compared to the other genotypes, a trend was observed for higher insulin levels among subjects bearing the PPAR α V162 allele who were noncarriers of the PPAR γ_2 A12 allele. These results suggest that carriers of the PPAR α V162 allele have higher insulin levels after a glucose load except when they also carry the PPAR γ_2 A12 allele. In contrast, C-peptide levels at some time points and the acute response to the glucose challenge were different between the genotypes (Fig. 1C). Indeed, similar C-peptide responses were observed for both genotypes that are noncarriers of the PPAR α V162 allele. However, distinct C-peptide responses were observed between PPAR γ_2 P12A genotypes under a PPAR α V162 background. These results suggest that the PPAR γ_2 P12A polymorphism has an effect on C-peptide levels, but only when the PPAR α V162 allele is present.

To evaluate whether the interaction between PPAR γ_2 P12A and PPAR α L162V polymorphisms was significant, we contrasted the four genotype groups for an interaction effect. The independent effect of each polymorphism and their interaction are summarized in Table 2. In this model, PPAR α L162V was not independently associated with plasma glucose, insulin, and C-peptide concentrations in the fasting state and in response to the OGTT. In contrast, PPAR γ_2 A12 allele was associated with lower acute insulin and C-peptide levels. In addition, the interaction between the two genes was statistically significant for the acute and total C-peptide AUC during the OGTT. Fig. 2 presents the interaction effect for the acute C-peptide AUC. The PPAR γ_2 A12 allele was associated with lower C-peptide levels, but only in the presence of the PPAR α V162 allele. Thus, the effect of one allele in one gene seems to depend upon the presence of another allele in a second gene.

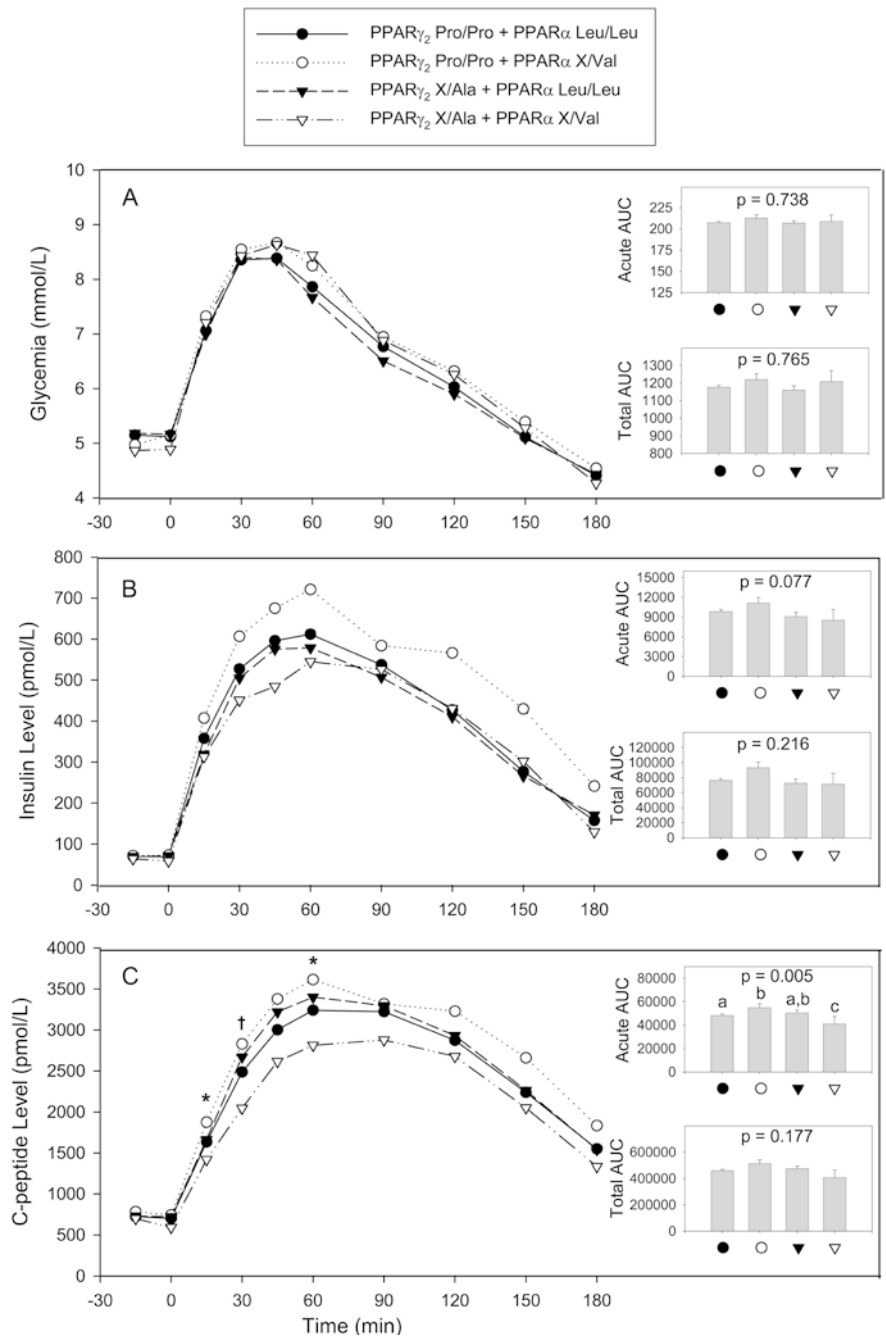
Discussion

The present study investigated the independent effect of PPAR γ_2 P12A and PPAR α L162V polymorphisms as well as their interaction on glucose, insulin, and C-peptide levels in the fasting state and following an OGTT. We showed that carriers of the PPAR α V162 allele had higher insulin and C-peptide levels in response to a glucose challenge compared to those homozygous for the L162 allele. In contrast, subjects classified on the basis of the PPAR γ_2 P12A polymorphism did not differ in terms of glucose and insulin phenotypes. Dividing subjects into four genotype combinations defined by the absence or presence of both variant alleles revealed significant differences between groups. Carriers of the

Table 1 Independent effects of the PPAR γ_2 P12A and PPAR α L162V polymorphisms. Values are geometric means [95% CI] (adjusted for age, gender and BMI). AUC area under the curve

Variables	PPAR γ_2 P12A			PPAR α L162V		
	Pro/Pro (n)	X/Ala (n)	<i>p</i> value	Leu/Leu (n)	X/Val (n)	<i>p</i> value
Fasting glucose (mmol/l)	4.95 [4.86; 5.05] (464)	4.94 [4.83; 5.05] (118)	0.764	5.19 [5.06; 5.31] (509)	5.06 [4.86; 5.26] (86)	0.155
Fasting insulin (pmol/l)	70.1 [62.4; 78.7] (457)	68.8 [58.9; 80.5] (118)	0.826	63.1 [56.2; 70.9] (504)	61.9 [50.2; 76.3] (85)	0.854
Fasting C-peptide (pmol/l)	656 [614; 702] (435)	657 [602; 716] (108)	0.991	679 [630; 731] (478)	695 [614; 787] (79)	0.617
Acute glucose AUC (mmol/l·30 min)	206 [203; 209] (412)	204 [199; 210] (101)	0.56	213 [210; 215] (464)	217 [212; 223] (67)	0.123
Acute insulin AUC (nmol/l·30 min)	8.24 [7.61; 8.92] (405)	7.70 [6.89; 8.60] (101)	0.216	8.50 [7.86; 9.20] (459)	9.75 [8.38; 11.35] (66)	0.062
Acute C-peptide AUC (nmol/l·30 min)	44.2 [42.0; 46.6] (401)	44.3 [41.6; 47.3] (100)	0.942	46.7 [44.7; 48.8] (453)	49.6 [46.1; 53.3] (66)	0.076
Total glucose AUC (mmol/l·180 min)	1188 [1127; 1252] (410)	1180 [1111; 1253] (101)	0.722	1231 [1177; 1287] (461)	1259 [1184; 1338] (67)	0.361
Total insulin AUC (nmol/l·180 min)	65.8 [61.2; 70.7] (403)	61.8 [55.6; 68.6] (101)	0.168	62.3 [57.8; 67.2] (456)	71.4 [62.7; 81.2] (66)	0.022
Total C-peptide AUC (nmol/l·180 min)	439 [424; 455] (392)	434 [410; 460] (99)	0.705	442 [426; 459] (444)	464 [437; 493] (65)	0.094

Fig. 1A–C Glucose (A), insulin (B) and C-peptide (C) levels during the oral glucose tolerance test (OGTT). Subjects have been divided into four groups according to the presence or absence of PPAR γ_2 P12A and PPAR α L162V mutations. Heterozygous and homozygous carriers of the variant allele were grouped together for both genes and are referred to as PPAR γ_2 X/Ala and PPAR α X/Val. Values are least-squared means adjusted for age, gender, and logBMI. Error bars on vertical histograms are standard errors. Differences between groups were tested using the MIXED procedure for each time point during the OGTT and for the acute and the total areas under the curve (AUC). Groups with similar letters above the bar do not differ significantly. *Group (○) differ from group (●) and (▽); † group (▽) differ from group (●), (○) and (▼) and group (○) differ from group (●)



PPAR α V162 allele had higher levels of insulin and C-peptide during the OGTT except when they carry the PPAR γ_2 A12 allele. Furthermore, the PPAR γ_2 A12 allele decreased C-peptide concentrations, but only on a PPAR α V162 genetic background.

Although the effect of the PPAR γ_2 P12A polymorphism on glucose and insulin homeostasis has been extensively studied (Stumvoll and Haring 2002a), this study is the first to demonstrate that the PPAR α V162 allele increases insulin and C-peptide levels during a glucose challenge. In vivo experiments using mice models have clearly confirmed the participation of PPAR α to maintain blood glucose during an acute

metabolic stress (Kersten et al. 1999; Leone et al. 1999). Indeed, PPAR α -null mice developed severe hypoglycemia when fasted. In addition, PPAR α -null mice are protected from a high-fat diet-induced insulin resistance (Guerre-Millo et al. 2001). This observation is consistent with present findings suggesting higher insulin and C-peptide levels among carriers of the PPAR α gene product with the greater transcriptional activity (V162 allele) (Flavell et al. 2000; Sapone et al. 2000). On the other hand, PPAR α activation with fibrates in rodent models of high-fat diet-induced insulin resistance and in genetic models of insulin resistance markedly improved their condition (Guerre-Millo et al. 2000). The genetic

Table 2 Effects of the PPAR_γ2 P12A, PPAR_α L162V, and their interaction. Values are geometric means [95% CI] (adjusted for age, gender and BMI). AUC area under the curve

	PPAR _γ 2 P12A/PPAR _α L162V						<i>p</i> value ^a	Interaction
	PPAR _γ 2 Pro/Pro PPAR _α Leu/Leu (<i>n</i>)	PPAR _γ 2 Pro/Pro PPAR _α X/Val (<i>n</i>)	PPAR _γ 2 X/Ala PPAR _α Leu/Leu (<i>n</i>)	PPAR _γ 2 X/Ala PPAR _α X/Val (<i>n</i>)	PPAR _γ 2 P12A	PPAR _α L162V		
Fasting glucose (mmol/l)	4.96 [4.85; 5.08] (379)	4.89 [4.68; 5.11] (61)	4.97 [4.84; 5.11] (96)	4.77 [4.52; 5.02] (17)	0.465	0.114	0.379	
Fasting insulin (pmol/l)	69.5 [61.2; 78.9] (374)	66.4 [52.1; 84.5] (60)	68.0 [56.4; 81.8] (96)	64.7 [47.5; 88.0] (17)	0.827	0.689	0.987	
Fasting C-peptide (pmol/l)	637 [590; 688] (356)	665 [603; 732] (56)	653 [589; 723] (88)	596 [498; 712] (15)	0.413	0.685	0.203	
Acute glucose AUC (mmol/l·30 min)	208 [202; 213] (346)	212 [204; 220] (47)	206 [198; 214] (86)	207 [189; 226] (12)	0.496	0.636	0.731	
Acute insulin AUC (nmol/l·30 min)	8.27 [7.20; 9.50] (341)	9.55 [7.98; 11.43] (46)	7.87 [6.65; 9.30] (86)	6.82 [4.94; 9.42] (12)	0.024	0.991	0.101	
Acute C-peptide AUC (nmol/l·30 min)	44.3 [40.1; 48.9] (336)	52.0 [44.6; 60.7] (46)	46.6 [41.5; 52.2] (85)	38.3 [32.6; 44.9] (12)	0.011	0.776	0.0008	
Total glucose AUC (mmol/l·180 min)	1199 [1127; 1275] (344)	1214 [1127; 1309] (47)	1182 [1098; 1271] (86)	1234 [1124; 1355] (12)	0.969	0.315	0.524	
Total insulin AUC (nmol/l·180 min)	65.5 [57.3; 75.0] (339)	74.0 [61.5; 89.1] (46)	61.9 [52.2; 73.5] (86)	60.0 [45.4; 79.4] (12)	0.079	0.591	0.323	
Total C-peptide AUC (nmol/l·180 min)	442 [405; 482] (329)	504 [439; 578] (45)	447 [400; 499] (84)	412 [355; 478] (12)	0.071	0.635	0.050	

^a *p* values are from the MIXED procedure in which the three independent variables are PPAR_γ2 P12A (Pro/Pro or X/Ala), PPAR_α L162V (Leu/Leu or X/Val) and the interaction (PPAR_γ2 P12A X PPAR_α L162V). Age, gender, and logBMI were also included in the model

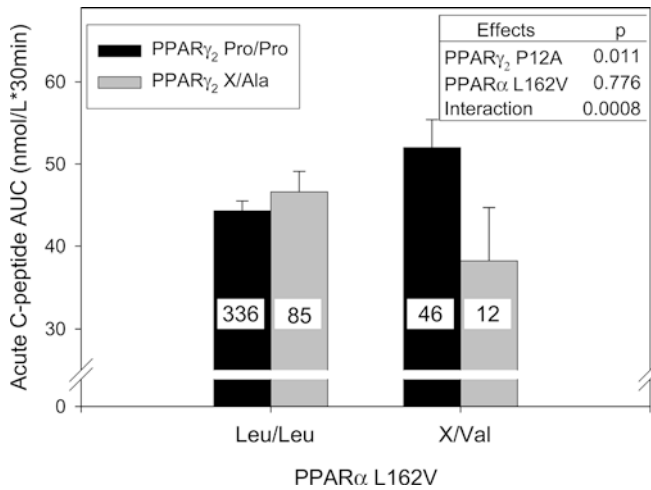


Fig. 2 Interactions between PPAR γ_2 P12A and PPAR α L162V polymorphisms for the acute C-peptide area under the curve (AUC) following the oral glucose tolerance test (OGTT). Bars represent the adjusted geometric mean \pm SE for each of the four genotype groups divided according to the presence or absence of PPAR γ_2 P12A and PPAR α L162V variants

advantage of PPAR α deficiency on insulin resistance and the favorable effect of its activation by fibrates therapy is analogous to the paradox observed with PPAR γ , with both heterozygous PPAR γ deficiency and PPAR γ agonist treatment improving insulin sensitivity (Walczak and Tontonoz 2002).

With similar ligands and overlapping functions, gene-gene interactions between PPAR isotypes should not be surprising. In fact, evidence is beginning to emerge in the literature suggesting that epistasis is probably more common than previously thought for glucose-related and insulin-related phenotypes (Bruning et al. 1997; Hsueh et al. 2001; Savage et al. 2002; Stumvoll et al. 2002c). However, providing functional data in support of our results is difficult. The PPAR γ_2 P12A and PPAR α L162V polymorphisms have opposite effects on the transcriptional activity of their respective receptor. Indeed, the A12 allele results in a less active form of PPAR γ_2 (Deeb et al. 1998) while the V162 allele results in a more active form of PPAR α (Flavell et al. 2000; Sapone et al. 2000). The observation that the PPAR γ_2 A12 allele is associated with lower insulin and C-peptide levels corroborates the phenotype observed in PPAR γ deficient mice (heterozygous PPAR γ $-/+$ mice) (Miles et al. 2000). However, the observation that the PPAR γ_2 A12 allele mediates its lowering effect only on a PPAR α genetic background complicates the explanation. One possible explanation may come from results obtained by Stumvoll et al. (2002c) who demonstrated that the PPAR γ_2 A12 allele was associated with greater insulin sensitivity, but only in subjects carrying a polymorphism in the gene encoding IRS-1, which had been associated with diabetes. They suggested that the A12 allele of PPAR γ_2 becomes particularly advantageous on the background of a disadvantageous genetic polymorphism

in a second gene. Similarly, V162 allele in PPAR α impacts negatively on glucose and insulin homeostasis in our study, and the A12 allele in PPAR γ_2 attenuates the effect of the PPAR α V162 allele. This hypothesis is also consistent with the greater insulin sensitivity, measured by euglycemic-hyperinsulinemic glucose-clamp reported only in a subgroup of obese subjects carrying the A12 allele (Koch et al. 1999). Taken together, these observations suggest that the A12 allele increase insulin sensitivity, but only in a metabolically challenging milieu. On the other hand, evidences in the literature suggests that the A12 allele may impair insulin secretion under particular metabolic challenges, such as lipid infusion (Stefan et al. 2001) and type 2 diabetes (Mori et al. 2001). Accordingly, the metabolic stress caused by the L162V polymorphism may reduce insulin secretion in subjects carrying the A12 allele and explain the decrease in C-peptide levels observed in subjects carrying both variants. However, similar levels of glucose among genotype groups make this last mechanism unlikely and suggest a greater insulin sensitivity among carriers of the A12 allele rather than an impairment in insulin secretion.

The effect of the PPAR γ_2 P12A polymorphism on type 2 diabetes and related phenotypes is controversial. For example, while the PPAR γ_2 A12 allele was associated with a decreased risk of type 2 diabetes in Caucasians (Altshuler et al. 2000), the same allele was associated with an increased susceptibility to type 2 diabetes in the Oji-Cree population (Hegele et al. 2000). The effect of this polymorphism is also inconsistent regarding obesity and related phenotypes. Some studies suggested that the A12 allele is associated with lower adiposity caused by decreased adipogenesis (Deeb et al. 1998), while others claimed that this allele increases adiposity as a result of reduced lipolysis (Stumvoll and Haring 2002b). The discrepancy between studies may reflect the complex relationships between the PPAR γ_2 P12A polymorphism and metabolic traits. Gene-gene interactions may explain the discrepancy among association studies (Hirschhorn et al. 2002). Thus, results of the present study may partly explain the controversy surrounding the PPAR γ_2 P12A polymorphism and type-2-diabetes-related phenotypes.

Evaluating the effect of two polymorphisms in addition to their interactions on several variables related to glucose and insulin homeostasis will inevitably lead to multiple testing. We did not adjust p values for the number of tests reported because even if numerous variables were used, all were related to the same phenotype. However, it worth mentioning that the interaction observed between the two variants for the acute C-peptide levels remained largely significant, even after Bonferroni correction ($p=0.0072$). Nevertheless, because of the multiple testing nature of this study, the results should be interpreted with caution and require replication. The study should therefore be considered as exploratory generating hypotheses rather than testing hypotheses.

In conclusion, we observed a deleterious effect of the PPAR α V162 allele on glucose and insulin levels during a glucose challenge. In addition, results of the present study suggest that PPAR γ ₂ P12A and PPAR α L162V polymorphisms interact with each other to modulate some features of glucose and insulin homeostasis. A replication of this study is, however, required before a firm conclusion can be reached. The present study demonstrated that genetic polymorphisms in candidate genes encoding proteins with overlapping functions can interact and make a substantial contribution to the final manifestation of the trait. It also confirmed the importance to take into consideration gene-gene interactions in the genetic dissection of complex metabolic phenotypes.

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