Original Article

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

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Although cross-sectional studies have associated the Pro12Ala polymorphism of *PPARG* with type 2 diabetes, prospective studies offer more opportunities to investigate genetic variants. Associations between *PPARG* polymorphisms with insulin resistance parameters and with the 6-year incidence of impaired fasting glucose or type 2 diabetes were tested in 3,914 French Caucasians from the DESIR (Data From an Epidemiological Study on the Insulin Resistance Syndrome) cohort. In subjects normoglycemic at baseline (n = 3,498), the 6-year risk of hyperglycemia was lower in *PPARG* Ala carriers (odds ratio [OR] vs. ProPro = 0.66 [95% CI 0.44–0.99], P = 0.046 adjusted for sex, age, and BMI). Similar results were found with the *PPARG* C1431T single nucleotide polymorphism (SNP; adjusted OR = 0.65 [0.44–0.96], P = 0.036). Both alleles are in strong linkage disequilibrium (D′ = 0.669, P < 0.001). The baseline mean fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR) were lower in Ala carriers compared with ProPro homozygotes (P = 0.001 for both), with smaller increases in mean insulin and HOMA-IR during follow-up (P = 0.007 and 0.018, respectively). No association with insulin levels or HOMA-IR was found with C1431T. In this cohort, the *APM1* G-11391A SNP is associated with the development of hyperglycemia. The combined effects of *PPARG* Pro12Ala and *APM1* G-11391A SNPs showed no interaction on the risk of 6-year hyperglycemia. The *PPARG* Ala allele showed a relatively high protective effect in developing hyperglycemia and hyperinsulinemia during a 6-year period. Cumulative rather than synergistic effects of *PPARG* Pro12Ala and *APM1* SNPs on diabetes risk are suggested. *Diabetes* 55: 1157–1162, 2006

Peroxisome proliferator–activated receptor (PPAR)γ, a member of the nuclear hormone receptor subfamily of transcription factors, is involved in the expression of target genes implicated in adipocyte differentiation and glucose homeostasis (1). The gene encoding PPARγ (*PPARG*) is therefore a candidate for type 2 diabetes and obesity. PPARγ2, one of the two isoforms of PPARγ resulting from mRNA alternative splicing, is expressed at higher levels in adipose tissue. A C-to-G transversion in *PPARG* has been identified, resulting in a Pro12-to-Ala (Pro12Ala) substitution (2). The allele frequency of the Ala12 variant ranges from 0.12 in Caucasian Americans to 0.01 in Chinese populations (1). In some but not all cross-sectional studies, the Ala12 allele was less frequent among patients with type 2 diabetes compared with healthy control subjects (1,3,4). However, in the massively obese, the Pro12Ala polymorphism of *PPARG* was not associated with type 2 diabetes (5). Nevertheless, a meta-analysis (6) showed a modest but significant decrease in diabetes risk associated with the rare Ala allele. Another polymorphism, the C1431T silent substitution in the 6th exon of *PPARG*, has been shown to modulate the effect of Pro12Ala on susceptibility to type 2 diabetes (7).

Adiponectin, a plasmatic protein secreted specifically by adipose tissue, improves insulin sensitivity (8). A marked increase in plasma adiponectin levels in subjects treated with the PPARγ agonists thiazolidinediones has been

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Received for publication 26 May 2005 and accepted in revised form 3 January 2006.

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DESIR, Data From an Epidemiological Study on the Insulin Resistance Syndrome; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; PPAR, peroxisome proliferator–activated receptor; SNP, single nucleotide polymorphism.

TABLE 1
PPARG Pro12Ala genotype frequencies, according to glycemic status after 6 years in subjects who were normoglycemic at baseline: the DESIR study

	ProPro	ProAla	AlaAla
Normoglycemic	2,622 (79.4)	653 (19.8)	27 (0.8)
IFG	129 (84.9)	22 (14.5)	1 (0.6)
Type 2 diabetes	38 (84.2)	6 (15.8)	0 (0)
Hyperglycemia (IFG + type 2 diabetes)	167 (85.2)	28 (14.3)	1 (0.5)
OR for hyperglycemia (95% CI)*	1	0.67 (0.44–1.01)†	0.44 (0.06–3.35)‡
OR for hyperglycemia (95% CI)* (Ala+ vs. ProPro)	1	0.66 (0.44–0.99)§	

Data are *n* (%), unless otherwise indicated. *By multiple logistic regression analyses (adjustment for sex, age, and BMI). Codominant model (number of Ala alleles, 0, 1, 2, considered as a continuous variable): OR for hyperglycemia (95% CI) = 0.67 (0.45–0.99), $P = 0.043$. †$P = 0.057$; ‡$P = 0.428$; §$P = 0.046$.

observed. In a previous study, we showed that single nucleotide polymorphisms (SNPs) of the adiponectin gene (*APM1*) were associated with the development of hyperglycemia (9).

Prospective studies offer the opportunity to assess the long-term effect of developing type 2 diabetes of gene variants that have been previously associated with the disease in case/control studies. Our aim was to examine whether the Pro12Ala polymorphism of *PPARG* is associated with the incidence of impaired fasting glucose (IFG) or type 2 diabetes and with insulin resistance parameters in a large phenotypically well-characterized French general population with a 6-year follow-up. We also tested a potential interaction between the Pro12Ala polymorphism and *PPARG* C1431T and with other SNPs of *APM1*, based on the potential role of PPARγ in the regulation of adiponectin expression.

RESEARCH DESIGN AND METHODS

The study population, men and women aged 30–64 years, participated in the cohort for the Data From an Epidemiological Study on the Insulin Resistance Syndrome (DESIR), a 9-year follow-up study that aims to clarify the development of the insulin resistance syndrome (10). Participants were recruited from volunteers insured by the French social security system, which offers periodic health examinations free of charge. Subjects came from 10 health examination centers in the western central part of France. All subjects signed an informed consent. The protocol was approved by the consultative committee for the protection of subjects for biomedical research of Bicêtre Hospital. A total of 5,096 subjects genotyped for Pro12Ala had data available at baseline. Among them, 4,521 subjects were normoglycemic, of whom 3,498 could be followed for incident IFG and diabetes during a 6-year period (response rate 0.77).

Three classes of glycemic status were defined according to 1997 American Diabetes Association criteria (11): normoglycemia, defined as fasting plasma glucose <6.1 mmol/l; IFG, defined as fasting plasma glucose between 6.1 and 6.9 mmol/l; and type 2 diabetes, defined as fasting plasma glucose ≥7.0 mmol/l and/or treatment by antidiabetic agents.

Measurements. Weight, height, and waist circumferences were measured by trained personnel, and BMI (kg/m^2) was calculated. Venous blood samples were collected in the morning after subjects had fasted 12 h. Fasting plasma glucose was assayed by the glucose oxidase method applied to fluoro-oxalated plasma, using a Technicon RA 1000 (Bayer, Puteaux, France) or a Kone Automate (Evry, France); fasting serum insulin was measured by an enzymoimmunoassay with IMX (Abbott, Rungis, France) (12). To estimate peripheral insulin resistance, we used the homeostasis model assessment of insulin resistance (HOMA-IR), defined as the product of fasting serum insulin and glucose divided by 22.5 (13). The HOMA-IR is correlated with insulin resistance as assessed by a euglycemic-hyperinsulinemic clamp (14).

Genotyping. The Pro12Ala SNP was genotyped using an AOD (assay on demand) kit (Applied Biosystems). The PCR was performed with a GeneAmp 9700 PCR system. The conditions for the TaqMan reaction were 95°C for 10 s and 40 cycles of 92°C for 15 s, 60°C for 1 min, and 15°C for 5 s. C1431T SNP genotyping was performed by Applied Biosystems SNPlex technology based on a oligonucleotide ligation assay combined with multiplex PCR target amplification (http://www.appliedbiosystems.com). Allelic discrimination was performed through capillary electrophoresis analysis, using an Applied Biosystems 3730xl DNA analyzer and GeneMapper3.7 software. The genotypes were determined with an ABI PRISM 7900 HT sequence detection system. Of the subjects genotyped for Pro12Ala, ~100 could not be genotyped for C1431T. Adiponectin genotyping for adiponectin SNPs (G-11391A, C-11377G, T45G, and G276T) was described in our previous study (9).

Statistical analysis. Differences between genotype frequencies for glycemic status were compared by χ^2 tests. Adjusted odds ratios (ORs) associated with genotypes were calculated by multivariate logistic regression. For all SNPs, the number of minor alleles (0,1, and 2) was used as a continuous variable to test the codominant model. The associations of genotypes with continuous parameters were tested by ANOVA or ANCOVA for repeated measures, with adjustments for baseline age, BMI, and sex. Skewed variables (BMI, insulin, and HOMA-IR) were log transformed before statistical analyses. All statistics used Systat for Windows software (version 10).

Haplotype frequencies were estimated, using EH (estimating haplotype) frequencies software (available from ftp://linkage.rockefeller.edu/software/eh). Differences in haplotype frequencies between phenotypes were also calculated with this software (maximum likelihood method). Linkage disequilibriums were calculated with a two-locus linkage disequilibrium calculator (available from http://www.iop.kcl.ac.uk/IoP/Departments/PsychMed/GEpiBSt/software.shtml).

RESULTS

The genotypic distribution of the Pro12Ala polymorphism was in Hardy-Weinberg equilibrium (ProPro 80.1%, ProAla 19.0%, and AlaAla 0.9%). The Ala frequency was 10% in accord with previously described data in European Caucasian populations (1). The genotypic distribution of C1431T SNP was also in Hardy-Weinberg equilibrium (CC 78.3%, CT 20.4%, TT 1.4%, and T allelic frequency 12%). Genotype distributions of the different *APM1* SNPs are described elsewhere (9).

In the whole population, at baseline, the proportion of Ala carriers was lower (15.8%) in subjects with type 2 diabetes than in normoglycemic subjects (20.1%), but this difference was not significant because the number of diabetic subjects was small (data not shown). Among subjects normoglycemic at baseline, the frequency of Ala carriers was 14.8% in those who developed hyperglycemia (IFG + type 2 diabetes), significantly lower than the 20.6% who remained normoglycemic (OR adjusted for sex, age, and BMI = 0.66 [95% CI 0.44–0.99], $P = 0.046$) (Table 1). There were no significant interactions between genotype and sex, age, or baseline BMI on the risk of developing hyperglycemia during the follow-up (tested by logistic regression, data not shown). Similar results were found with the C1431T SNP: the T allele was less frequent in subjects who developed hyperglycemia (16.1%) when compared with those who remained normoglycemic (22.4%; multiply adjusted OR = 0.65 [0.44–0.96], $P = 0.036$) (Table 2). Both polymorphisms were in strong linkage disequilib-

TABLE 2
PPARG C1431T genotype frequencies, according to glycemic status after 6 years in subjects who were normoglycemic at baseline: the DESIR study

	CC	CT	TT
Normoglycemic	2,493 (77.6)	673 (20.9)	47 (1.5)
IFG	125 (83.3)	24 (16.0)	1 (0.7)
Type 2 diabetes	37 (86.0)	6 (14.0)	0 (0)
Hyperglycemia (IFG + type 2 diabetes)	162 (84.0)	30 (15.5)	1 (0.5)
OR for hyperglycemia (95% CI)*	1	0.68 (0.45–1.02)†	0.28 (0.04–2.09)‡
OR for hyperglycemia (95% CI)* (T+ vs. CC)	1	0.65 (0.44–0.97)§	

Data are *n* (%), unless otherwise indicated. *By multiple logistic regression analyses (adjustment for sex, age, BMI). Codominant model (number of T alleles: 0, 1, 2, considered as a continuous variable): OR for hyperglycemia (95% CI) = 0.65 (0.45–0.95), $P = 0.027$. †$P = 0.062$; ‡$P = 0.428$; §$P = 0.036$.

rium ($D' = 0.669$, $P < 0.001$). The distribution of haplotypes was significantly associated with the incidence of hyperglycemia (Table 3). The analysis by logistic regression, using true genotype combinations instead of calculated haplotypes, showed the same results (Table 3). No significant interaction between Pro12Ala and C1431T genotypes was detected by logistic regression.

To assess effects on insulin resistance before the onset of hyperglycemia, we analyzed continuous traits according to genotypes in subjects normoglycemic at entry who remained normoglycemic after 6 years. Fasting insulin and HOMA-IR were lower in Ala carriers ($P = 0.001$ for both variables, adjusted for sex, age, and BMI) (Table 4). The effect of the *PPARG* Pro12Ala genotype on the differences between baseline and 6-year insulin and HOMA-IR (genotype × time interaction) were significant for fasting insulin ($P = 0.007$) and HOMA-IR ($P = 0.018$). The increases in fasting insulin and HOMA-IR during follow-up were higher in the ProPro homozygotes than in the Ala carriers, even when further adjusted for baseline values. Because no associations were found with the C1431T polymorphism, we only analyzed Pro12Ala for interaction analyses with *APM1* SNPs.

Among the four adiponectin SNPs we previously genotyped (9), only G-11391A was significantly associated with hyperglycemia at T6 (reported here for the first time). Among subjects normoglycemic at baseline, −11391A carriers had an increased risk of hyperglycemia at 6 years compared with GG subjects (OR adjusted for sex, age, BMI) (Table 5). This result remained unchanged when further adjusted for BMI variation at follow-up (OR = 1.55 [1.09–2.19], $P = 0.014$; or after inclusion of Pro12Ala in the model: 1.56 [1.10–2.21], $P = 0.013$). The interaction between the *PPARG* Pro12Ala polymorphism and the *APM1* G-11391A polymorphism on the risk of hyperglycemia at 6 years was not significant ($P = 0.21$). When compared with the most frequent genotype combination GG/ProPro, the combination of both protective genotypes (GG/Ala+) significantly decreased the risk of hyperglycemia (0.60 [0.38–0.98], adjusted for sex, age and BMI; $P = 0.04$) (Table 7), whereas the association of both deleterious genotypes (A+/ProPro) significantly increased the risk (1.53 [1.03–2.25], $P = 0.033$). Taking as the reference group the most "deleterious" combination for both polymorphisms (A+/ProPro), the combination of the protective genotypes (GG/Ala+) decreased the risk significantly (0.56 [0.38–0.81], adjusted for sex, age, and BMI; $P = 0.003$) (Table 6).

DISCUSSION

The ACDC/adiponectin SNPs had already been shown to contribute to the development of impaired glucose homeostasis over a 3-year period in the prospective DESIR cohort (9). In the current study, we have shown that the Ala allele of the *PPARG* Pro12Ala polymorphism is protective from the appearance of mild fasting hyperglycemia and type 2 diabetes during a 6-year follow-up in subjects normoglycemic at baseline. The *PPARG* exon 6 C1431T SNP is also associated with a lower risk. The *APM1* G-11391A SNP has also been shown to be associated with the onset of hyperglycemia in the same period. Furthermore, the effects of Pro12Ala and *APM1* G-11391A SNPs on the risk of hyperglycemia appear to be cumulative. The Ala allele also had a protective effect on the deterioration of both fasting insulin and HOMA-IR over this period, indicating the main effect of *PPARG* Pro12Ala may be on insulin resistance.

Although the modulation of insulin sensitivity associated with the Ala allele is well established in cross-sectional studies, it is the first time, to our knowledge, that a longitudinal effect has been found in a general Caucasian population. It is noteworthy that this effect appears even in the absence of impaired glucose levels; it was observed in normoglycemic subjects during a 6-year period (after exclusion of subjects with IFG or type 2 diabetes at baseline and/or 6 years from the analysis). In an African-

TABLE 3
PPARG haplotype frequencies according to the prospective risk of hyperglycemia

	Hyperglycemia	Normoglycemia	Statistics
Pro-C	0.901	0.849	Versus all: $\chi^2 = 7.82$, $df = 1$, $P = 0.005$
Pro-T	0.021	0.044	Versus ProC: $\chi^2 = 5.18$, $df = 1$, $P = 0.023$
Ala-C	0.016	0.032	Versus ProC: $\chi^2 = 3.17$, $df = 1$, $P = 0.075$
Ala-T	0.062	0.076	Versus ProC: $\chi^2 = 1.50$, $df = 1$, $P = 0.221$

Global $\chi^2 = 10.80$, $df = 3$, $0.01 < P < 0.025$. Logistic regression analysis of hyperglycemia risk using genotype combinations: Ala+/T + vs. ProPro/CC: OR (95% CI) = 0.709 (1.111–0.453), $P = 0.133$. Ala+/CC vs. ProPro/CC: 0.482 (1.103–0.210), $P = 0.084$. ProPro/T + vs. ProPro/CC: 0.490 (1.010–0.238), $P = 0.053$.

TABLE 4
Characteristics of subjects normoglycemic at baseline who remained free of hyperglycemia (IFG or type 2 diabetes) at 6 years according to *PPARG* genotypes

	Pro12Ala				C1431T			
	ProPro	Ala+	*P* genotype	*P* interaction genotype time	CC	T+	*P* genotype	*P* interaction genotype time
n	2,622	680	—	—	2,491	720	—	—
Fasting glucose (mmol/l)								
Baseline	5.15 ± 0.01	5.13 ± 0.02			5.15 ± 0.01	5.13 ± 0.02		
6 years	5.16 ± 0.01	5.17 ± 0.03	0.525	0.263	5.17 ± 0.01	5.17 ± 0.02	0.736	0.658
Fasting insulin (mU/l)								
Baseline	5.30 (5.21–5.40)	5.15 (4.98–5.33)			5.29 (5.19–5.38)	5.22 (5.04–5.40)		
6 years	6.00 (5.88–6.12)	5.50 (5.28–5.72)	0.001	0.007	5.94 (5.81–6.07)	5.70 (5.47–5.93)	0.193	0.260
HOMA-IR								
Baseline	1.21 (1.19–1.23)	1.17 (1.13–1.22)			1.21 (1.18–1.23)	1.20 (1.15–1.24)		
6 years	1.38 (1.35–1.41)	1.26 (1.21–1.31)	0.001	0.018	1.36 (1.33–1.39)	1.31 (1.25–1.36)	0.236	0.242

Data are means ± SE or geometric mean (95% CI). *P* values are from ANCOVA, adjusted for sex, BMI, and age at inclusion.

American population followed prospectively, Kao et al. (15) found no significant differences in the rate of change of insulin or glucose between ProPro and ProAla groups. Moreover, in this population, the effects of the *PPARG* Pro12Ala polymorphism on both HOMA-IR and fasting insulin at baseline were only seen for BMI <25 kg/m^2.

A meta-analysis of cross-sectional studies showed a lower risk of type 2 diabetes in Ala carriers, which is consistent with our results (6). Few prospective studies (15–17) have been published on the *PPARG* Pro12Ala polymorphism and the incidence of type 2 diabetes. In an African-American population sample, Kao et al. (15) reported no association with type 2 diabetes, but this is likely to be caused by the low Ala frequency in that population (~2%) and a lack of statistical power. In a nested case-control study in women from the Nurses' Health Study (16), the Ala allele was associated with a significantly decreased risk of developing type 2 diabetes, similar to our results. In this study, the BMI of Ala carriers (28.7 kg/m^2) was higher than in our population (24.4 kg/m^2). The effects of the *PPARG* SNP on mild hyperglycemia were not tested in these two studies. Lindi et al. (17) reported that the Ala allele predisposed to the development of type 2 diabetes in obese subjects with impaired glucose tolerance. In contrast, in a cross-sectional study in a French population, we recently found no significant effect of the Pro12Ala SNP on the genetic risk of type 2 diabetes in morbidly obese subjects (5). Therefore, the protective effect of Ala appears to only be seen in normoglycemic subjects drawn from general populations, as in our study. Interestingly, the Pro12Ala polymorphism was not associated with the incidence of type 2 diabetes in the DESIR subjects who were already IFG at baseline (data not shown). Thus, severe obesity and/or IFG could mask an eventual protective effect of the Ala allele. This could be caused by abnormalities in pancreatic β-cell function, which are already present in such subjects.

The T allele of *PPARG* C1431T SNP was also associated with a lower risk to develop hyperglycemia. Although both alleles are in strong linkage disequilibrium, they could act independently through different physiological mechanisms, since in our study, Pro12Ala was the only SNP associated with lower insulin levels, and this effect did not interact with C1431T.

Our study did not detect a significant interaction between SNPs in *APM1* and *PPARG* on hyperglycemia risk at 6 years. When combined, subjects with both protective genotypes (Ala12 and *APM1* GG-11391) had the lowest risk of hyperglycemia at 6 years. In women from a prospective nested case-control study, Hu et al. (18) found a lower risk of type 2 diabetes with the Ala12 allele and the adiponectin GG +276 genotype. They did not test G-11391A, an SNP associated with hyperglycemia in our French population (9). As already reported and discussed in our previous study (9), the G-11391A SNP effect is intriguing because

TABLE 5
APM1 G-11391A genotype frequencies, according to glycemic status after 6 years in subjects who were normoglycemic at baseline: the DESIR study

	GG	GA	AA
Normoglycemic	2,702 (82.2)	554 (16.9)	31 (0.9)
IFG	116 (76.3)	34 (22.4)	2 (1.3)
Type 2 diabetes	34 (77.3)	10 (22.7)	0 (0)
Hyperglycemia (IFG + type 2 diabetes)	150 (76.5)	44 (22.4)	2 (1.0)
OR for hyperglycemia (95% CI)*	1	1.62 (1.13–2.33)†	1.65 (0.38–7.13)‡
OR for hyperglycemia (95% CI)* (A+ vs. GG)	1	1.62 (1.14–2.31)	

Data are *n* (%), unless otherwise indicated. *By multiple logistic regression analyses (adjustment for sex, age, BMI). Codominant model (number of A alleles, 0, 1, 2, considered as a continuous variable): OR for hyperglycemia (95% CI) = 1.55 (1.12–2.14), $P = 0.008$. †$P = 0.008$; ‡$P = 0.505$; §$P = 0.007$.

TABLE 6
The association between combinations of *APM1* G-11391A and *PPARG* Pro12Ala and risk of hyperglycemia at 6 years in subjects normoglycemic at baseline: the DESIR study

	GG/ProPro	GG/Ala+	A+/Ala+	A+/ProPro
Normoglycemic	2,148 (65.3)	554 (16.9)	123 (3.7)	462 (14.1)
IFG	100 (65.8)	16 (10.5)	7 (4.6)	29 (19.1)
Type 2 diabetes	29 (65.9)	5 (11.3)	1 (2.3)	9 (20.5)
Hyperglycemia (IFG + type 2 diabetes)	129 (65.8)	21 (10.7)	8 (4.1)	38 (19.4)
OR (95% CI) for hyperglycemia*	0.78 (0.58–1.03)†	0.56 (0.38–0.81)‡	1.10 (0.61–1.96)§	1
OR (95% CI) for hyperglycemia*	1	0.60 (0.38–0.98)‖	1.33 (0.62–2.83)¶	1.53 (1.03–2.25)#

Data are *n* (%), unless otherwise indicated. *Adjusted for sex, age, and BMI; †$P = 0.082$; ‡$P = 0.003$; §$P = 0.760$; ‖$P = 0.040$; ¶$P = 0.460$; #$P = 0.033$.

the A allele has been shown to increase adiponectin concentration.

As expected in polygenic multifactorial diseases, it can be estimated that the predictive power of Pro12Ala SNP is low and could not be used for clinical purposes. For comparison, in the logistic regression analysis equation with Pro12Ala and G-11391A SNPs, ORs for hyperglycemia of 0.48 (95% CI 0.35–0.65, $P < 0.001$) and 1.21 (1.17–1.26, $P < 0.001$) were associated with female sex and with one BMI unit change, respectively (when BMI was taken into account, age had no effect on hyperglycemia risk). Nevertheless, as in all similar genetic epidemiological studies, the aim is to add to the physiopathological knowledge and to assess the involvement of a metabolic pathway in the morbid phenotype. It has been estimated from earlier cross-sectional studies that the Ala population-attributable risk was −25% (18). In other words, if the entire population carried the Ala allele, the prevalence for type 2 diabetes would be 25% lower. This result is very near to what we have calculated here because from our data the reduction would be 27.9%. As already discussed by Stumvoll and Häring (1), this underlines the importance of alleles with weak individual effect but high population prevalence, and it clearly indicates the prominent role of *PPARG* among candidate genes for common type 2 diabetes or impaired glucose metabolism.

These results show the protective effect of *PPARG* Ala allele on the risk of developing hyperglycemia and hyperinsulinemia during a 6-year follow-up and suggest cumulative rather than synergistic effects of Pro12Ala *PPARG* and G-11391A adiponectin SNPs on this risk.

ACKNOWLEDGMENTS

This work was supported by cooperative contracts between INSERM and the Caisse Nationale de l'Assurance Maladies des Travailleurs Salariés (CNAMTS; contract 3AM004) and Novartis Pharma (convention 98297). This work was also supported by INSERM Réseaux en Santé Publique (contracts 494003 and 4R001C), INSERM Interactions Entre les Déterminants de la Santé (contract 4D002D), the Association Française des Diabétiques (AFD), the Association Diabète Risque Vasculaire, the Centre de Recherche et d'Information Nutritionnelles (CERIN), the Fédération Française de Cardiologie, La Fondation de France, the Association de Langue Française pour l'Etude du Diabète et des Maladies Métaboliques (ALFEDIAM), the Office National Interprofessionnel des Vins (ONIVINS), Ardix Medical, Bayer Diagnostics, Becton Dickinson, Cardionics, Lipha Pharmaceuticals, Merck Santé, Novo Nordisk, Pierre Fabre, and Topcon.

APPENDIX

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REFERENCES

1. Stumvoll M, Häring H: The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism. *Diabetes* 51:2341–2347, 2002
2. Yen C-J, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR: Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Comm* 241:270–274, 1997
3. Malecki MT, Frey J, Klupa T, Skupien J, Walus M, Mlynarski W, Sieradzki J: The Pro12Ala polymorphism of PPARgamma2 gene and susceptibility to type 2 diabetes mellitus in a Polish population. *Diabetes Res Clin Pract* 62:105–111, 2003
4. Muller YL, Bogardus C, Beamer BA, Shuldiner AR, Baier LJ: A functional variant in the peroxisome proliferator–activated receptor gamma2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. *Diabetes* 52:1864–1871, 2003
5. Vasseur F, Helbecque N, Lobbens S, Vasseur-Delannoy V, Dina C, Clement K, Boutin P, Kadowaki T, Scherer PE, Froguel P: Hypoadiponectinemia and high risk of type diabetes P are associated with adiponectin-encoding (ACDC) gene promoter variants in morbid obesity evidence for a role of ACDC in diabesity. *Diabetologia* 48:892–899, 2005
6. Altshuler D, Hirschborn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
7. Doney ASF, Fischer B, Cecil JE, Boylan K, McGuigan, Ralston SH, Morris AD, Palmer CNA: Association of the Pro12Ala and C1431 variants of PPARG and their haplotypes with susceptibility to type 2 diabetes. *Diabetologia* 47:555–558, 2004
8. Stefan N, Stumvoll M: Adiponectin—its role in metabolism and beyond. *Horm Metab Res* 34:469–474, 2002
9. Fumeron F, Aubert R, Siddiq A, Betoulle D, Pean F, Hadjadj S, Tichet J, Wilpart E, Chesnier M-C, Balkau B, Froguel P, Marre M, for the Epidemiologic Data on the Insulin Resistance Syndrome (DESIR) Study Group: Adiponectin gene polymorphisms and adiponectin levels are independently associated with the development of hyperglycemia during a 3-year period: the epidemiologic data on the insulin resistance syndrome prospective study. *Diabetes* 53:1150–1157, 2004
10. Balkau B: [An epidemiologic survey from a network of French Health Examination Centre: (D.E.S.I.R.) Epidemiologic Data on the Insulin Resistance Syndrome]. *Rev Epidemiol Sante Publique* 44:373–375, 1996

11. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
12. Gallois Y, Vol S, Caces E, Balkau B: Distribution of fasting serum insulin measured by enzyme immunoassay in an unselected population of 4,032 individuals: reference values according to age and sex: D.E.S.I.R. Study Group: Donnees Epidemiologiques sur le Syndrome d'Insulino-Resistance. *Diabetes Metab* 22:427–431, 1996
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
14. Katsuki A, Sumida Y, Gabazza EC, Murashima S, Furuta M, Araki-Sasaki R, Hori Y, Yano Y, Adachi Y: Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. *Diabetes Care* 24:362–365, 2001
15. Kao WH, Coresh J, Shuldiner AR, Boerwinkle E, Bray MS, Brancati FL, Atherosclerosis Risk in Communities Study: Pro12Ala of the peroxisome proliferator–activated receptor-gamma2 gene is associated with lower serum insulin levels in nonobese African Americans: the Atherosclerosis Risk in Communities Study. *Diabetes* 52:1568–1572, 2003
16. Memisoglu A, Hu FB, Hankinson SE, Liu S, Meigs JB, Altshuler DM, Hunter DJ, Manson JE: Prospective study of the association between the proline to alanine codon 12 polymorphism in the PPARgamma gene and type 2 diabetes. *Diabetes Care* 26:2915–2917, 2003
17. Lindi VI, Uusitupa MI, Lindström J, Louheranta A, Eriksson JG, Valle TT Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Tuomilehto J, Finnish Diabetes Prevention Study: Association of the Pro12Ala polymorphism in the PPAR-gamma2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. *Diabetes* 51:2581–2586, 2002
18. Hu FB, Doria A, Li T, Meigs JB, Liu S, Memisoglu A, Hunter D, Manson JE: Genetic variation at the adiponectin locus and risk of type 2 diabetes in women. *Diabetes* 53:209–213, 2004