Association of the Pro12Ala and C1431T Variants of *PPARγ* and Their Haplotypes with Susceptibility to Gestational Diabetes

Barbara Heude, Veronique Pelloux, Anne Forhan, Jean-François Bedel, Jean-Marc Lacorte, Karine Clément, and Marie-Aline Charles, and the EDEN Mother-Child Cohort Study Group

Institut National de la Santé et de la Recherche Médicale (INSERM) (B.H., A.F., M.-A.C.), Centre de recherche en Epidémiologie et Santé des Populations Centre for Research in Epidemiology and Population Health, UMRS1018 Team 10, Lifelong Epidemiology of Diabetes, Obesity, and Chronic Kidney Disease, and Université Paris-Sud (B.H., A.F., M.-A.C.), Unité Mixte de Recherche en Santé (UMRS) 1018, F-94807 Villejuif, France; INSERM (V.P., J.-F.B., K.C.), U872, Team 7, Nutriomique, and University Pierre et Marie Curie-Paris 6 (V.P., J.-M.L., K.C.), Cordelier Research Center, UMRS 872, F-75006 Paris, France; and Assistance Publique/Hôpitaux de Paris (AP/HP) (V.P., J.-F.B., K.C.), Pitié Salpêtrière Hospital, Nutrition and Endocrinology Department, Research Center for Human Nutrition (CRNH, Ile de France), and Département de Biochimie Endocrinienne et Oncologique (J.-M.L.), Unité Fonctionnelle de Nutrigenetique, Hôpital Pitié-Salpêtrière, AP-HP, F-75013 Paris, France

Background: The protective role of the Ala allele in the Pro12Ala polymorphism of *PPARγ* on type 2 diabetes has been well established but not confirmed in the context of pregnancy, for gestational diabetes, a known predictor of later type 2 diabetes onset. Another *PPARγ* polymorphism, the C1431T, is in strong linkage disequilibrium with Pro12Ala and has been shown to be associated with body weight, but its association with diabetes is controversial.

Research Design and Methods: In 1708 women of the EDEN mother-child cohort, the *PPARγ* Pro12Ala and C1431T polymorphisms were genotyped and analyzed in association with maternal prepregnancy body mass index, obesity before pregnancy, and gestational diabetes, separately and also combined in haplotypes.

Results: The prevalence of obesity was significantly higher in mothers with the Ala/Ala genotype compared with carriers of the Pro allele (35 *vs.* 9%, $P < 0.0001$), but there was no cases of gestational diabetes in Ala/Ala mothers. Mothers homozygous for the T allele of C1431T were also more obese (24 *vs.* 9%, $P = 0.035$), and three times more had gestational diabetes (18 *vs.* 6%, $P = 0.044$). Frequencies of haplotypes for these two single-nucleotide polymorphisms differed significantly in mothers with and without gestational diabetes; in comparison with the Pro-C haplotype, the Pro-T haplotype conferred the highest risk [odds ratio (95% CI) = 1.89 (1.05–3.40)], and the Ala-C the lowest risk [odds ratio (95% CI) = 0.12 (0.52–1.70)].

Conclusions: These results from a haplotype analysis, show for the first time that genetic variations in the *PPARγ* gene could play a role in the susceptibility to develop gestational diabetes. **(*J Clin Endocrinol Metab* 96: E1656–E1660, 2011)**

Peroxisome proliferator-activated receptor γ (PPARγ) is a transcription factor with a key role in adipogenesis and insulin sensitization (1). Frequent mutations in the *PPARγ* gene have been described to be associated with obesity and diabetes-related phenotypes (2–4). The common structural polymorphism with a proline (Pro) to al-

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Abbreviations: BMI, Body mass index; CI, confidence interval; GDM, gestational diabetes mellitus; OR, odds ratio; PPARγ, peroxisome proliferator-activated receptor γ; SNP, single-nucleotide polymorphism.

TABLE 1. Frequencies of *PPARγ* Pro12Ala and C1431T in mothers and associations with maternal prepregnancy BMI, obesity, and GDM according to genotype: EDEN Mother-Child Cohort Study

	Pro12Ala				
	Pro/Pro	**Pro/Ala**	**Ala/Ala**	***P1*[a]**	***P2*[a]**
Genotype frequencies[b]	80% (1357)	19% (322)	1% (17)		
Maternal BMI before pregnancy (kg/m^2)	23.2 (4.6)	23.2 (4.6)	26.8 (5.7)	0.007	0.002
Obesity before pregnancy	9% (118)	8% (26)	35% (6)	0.003	<0.0001
GDM	7% (92)	5% (17)	0	0.25	
Adjusted[c] OR for obesity	1	0.93 (0.6–1.45)	6.32 (2.28–17.55)		
Adjusted[d] OR for GDM	1	0.73 (0.42–1.27)			

Data are % (n) or mean (SD).

[a] *P1* = *P* value for overall association (2 df); *P2* = *P* value for association under the recessive model (1 df).

[b] χ^2 (1 df)/*P* values for departure from Hardy-Weinberg equilibrium were 0.19/0.66 and 1.1/0.29, respectively, for Pro12Ala and C1431T.

[c] Adjusted for maternal age.

[d] Adjusted for maternal age and pre-pregnancy BMI.

anine (Ala) substitution has been identified as a functional variant. Compared with the Pro allele, the Ala allele associates with reduced activity of PPARγ (2). This polymorphism was extensively investigated for association with obesity and type 2 diabetes and is considered to be one of the best-replicated genetic risk factors of common type 2 diabetes, carrying the Ala variant being protective against type 2 diabetes (5–7). The association with adiposity level and obesity is not as consistent (8–10). An additional variant, the C1431T located in exon 6 of *PPARγ*, associates with the susceptibility to cardiovascular diseases (11, 12), leptin concentrations (4), and body mass index (BMI) (13, 14). These two polymorphisms are in close linkage disequilibrium (9, 13), and it has been suggested that they have an opposite association with body weight (3) but an additive protective effect against developing hyperglycemia (15). The effects of combinations of linked polymorphisms may help to explain some discrepancies between studies where only one of these variants is investigated.

Gestational diabetes mellitus (GDM) is defined as an abnormal glucose tolerance diagnosed for the first time during pregnancy (16) and is considered as an important predictor for later development of type 2 diabetes (17). In a recent review, Watanabe (18) acknowledged that very little is known about the genetic predisposition for GDM and that further studies are required to better understand its etiology.

We investigate the association of Pro12Ala and C1431T polymorphisms of the *PPARγ* gene, separately and combined in haplotypes, with GDM.

Materials and Methods

Population studied

The EDEN study (study of pre- and postnatal determinants of children's growth and development) is an ongoing mother-child cohort in two centers, Nancy and Poitiers (France), with a follow-up of the child until their fifth birthday. This study enrolled 2002 women during pregnancy and has previously been described in more detail (19). The study was approved by the ethics committee (Comités de consultation pour la protection des personnes se prêtant à la recherche biomédicale) of Kremlin Bicêtre and by the Data Protection Authority Comission Nationale de l'Informatique et des Libertés (CNIL).

Among the eligible women who agreed to participate, 1708 were included in the present analysis because they had information on BMI before pregnancy and GDM and had at least one of the two single-nucleotide polymorphisms (SNP) genotyped.

Measurements

At 24–28 wk, mothers had a clinical examination performed by midwife research assistants, and they completed a self-administered questionnaire. Maternal height was measured with a wall Seca 206 stadiometer (Hamburg, Germany) to the nearest 0.2 cm. Mothers came to the examination fasting and received a 50-g glucose load, and glucose concentrations were measured 1 h after the glucose challenge. Women with plasma glucose over 130 mg/dl in Nancy and 140 mg/dl in Poitiers were scheduled for a 100-g 3-h oral glucose tolerance test. Finally, clinical diagnoses of GDM were extracted from clinical records, and the difference in thresholds between centers did not result in different GDM prevalence.

Weight before pregnancy was obtained by interview at inclusion. Prepregnancy BMI was computed as reported weight (kilograms) divided by square of measured height (meters).

Genotyping

Genotyping used two devices. For the first 729 women included in the study with available DNA, the Pro12Ala polymorphism was determined using the LightCycler apparatus (Roche Diagnostics, Meylan, France) and hybridization probes. The design and synthesis of primers and probes were by TIB MOLBIOL (Berlin, Germany). The PCR contained 20 ng DNA, 1× Fast Start DNA master hybridization probes, 3 mM $MgCl_2$, 0.5 μM primers, and 0.15 μM probes in 10 μl total volume. SNP genotyping was carried out using melting curves analysis. The second part of the cohort (1024 mothers with available DNA) was genotyped on amplified DNA using TaqMan (Applied Biosystems, Foster City, CA). The probes were directly ordered from Applied

TABLE 1. Continued

C1431T				
C/C	C/T	T/T	*P1*[a]	*P2*[a]
78% (1285)	21% (340)	1% (17)		
23.2 (4.7)	23.6 (4.7)	24.3 (5.7)	0.29	0.35
8% (109)	10% (36)	24% (4)	0.05	0.035
6% (76)	6% (22)	18% (3)	0.12	0.044
1	1.28 (0.89–1.91)	3.5 (1.12–10.9)		
1	1.09 (0.66–1.81)	3.65 (0.98–13.62)		

Biosystems, and after synthesis, they were used according to the manufacturer's guidelines. The TaqMan assays were then read on a 7900HT Fast Real-Time PCR System (Applied Biosystems), and the alleles were called using the SDS software (Applied Biosystems). For C1431T, the whole cohort was genotyped using the latter device.

The two SNP had a genotyping call rate above 98%, and all of the duplicate controls (96 DNA analyzed with both devices) were fully concordant. Details for all genotyping primers, probes, and PCR conditions are available on request from the corresponding author.

The amplified DNA samples were amplified by PCR that includes one step of denaturation at 95 C for 10 min, followed by 40 cycles at 92 C for 15 sec and 60 C for 1 min. The concordance between genotypes of amplified and genomic DNA was checked on a 96-well plate and was 100% for both SNP.

Statistical analysis

Maternal BMI and prevalence of obesity before pregnancy and GDM were compared according to the maternal Pro12Ala and C1431T genotypes and statistical difference tested using χ^2 tests for percentages and Fisher tests for means. Tests under a recessive model (homozygous for the variant compared with others) were also performed. Odds ratios (OR) for obesity before pregnancy and GDM were calculated using logistic regression models adjusted for maternal age (and prepregnancy BMI for GDM).

Finally, the D′ (normalized linkage disequilibrium coefficient) and r^2 for linkage disequilibrium between the two SNP and the haplotype frequencies were calculated using the Thesias software (20). This software was also used to compare haplotype frequencies in mothers with and without GDM and to calculate OR of GDM for rarer haplotypes in comparison with the most common haplotype, adjusting for maternal age and prepregnancy BMI. The association of prepregnancy BMI with haplotypes was also analyzed with the Thesias software, but the results are not shown.

Other statistical analyses used Statistical Analysis Software (SAS, Cary, NC) version 9.

Results

Allele frequencies for the rare alleles of Pro12Ala and C1431T were, respectively, 10.4 and 11.4%, and there was no departure from Hardy-Weinberg equilibrium ($P = 0.66$ and 0.29, respectively, Table 1).

Mothers carrying the Ala/Ala genotype had higher BMI before pregnancy (26.8 ± 5.7 *vs.* 23.2 ± 4.6 kg/m^2, $P = 0.002$), higher prepregnancy obesity rates (35 *vs.* 9%, $P < 0.0001$), and lower prevalence of GDM because none of the Ala/Ala mothers developed diabetes (Table 1). Ala/Ala conferred an OR for obesity before pregnancy of 6.32 [95% confidence interval (CI) = 2.28–17.55], in comparison with Pro/Pro mothers, after adjusting for maternal age.

Mothers homozygous for the T allele of the C1431T SNP were significantly more obese (24 *vs.* 9%, $P = 0.035$) and also had higher prepregnancy BMI, although the difference was not statistically significant (Table 1). The prevalence of GDM was significantly higher in the T/T homozygotes (18 *vs.* 6%, $P = 0.044$). After adjustment for maternal age and prepregnancy BMI (for GDM), OR (95% CI) of obesity before pregnancy and GDM in homozygous for the T allele were, respectively, 3.50 (1.12–10.9) and 3.65 (0.98–13.62).

The Pro12Ala and C1431T polymorphisms were in linkage disequilibrium (D′ = 0.68 and r^2 = 0.42 in all mothers, Table 2). Haplotype frequencies are shown in Table 2, in all mothers and separately in mothers with and without GDM. Carrying the different haplotypes modified the risk of GDM; in comparison with the most frequent haplotype (Pro-C), the OR (95% CI) for GDM was 1.89 (1.05–3.40) in Pro-T carriers and 0.12 (0.02–0.75) in Ala-C carriers.

Discussion

The present study in a population of pregnant women showed that the variant of two frequent polymorphisms of the *PPARγ* gene, Pro12Ala and C1431T, were inversely

TABLE 2. Haplotype frequencies for the variants Pro12Ala and C1431T in all mothers and in mothers with or without GDM: EDEN Mother-Child Cohort Study

Haplotype	Frequency in all mothers (n = 1708) (%)	Frequency in mothers without GDM (n = 1609) (%)	Frequency in mothers with GDM (n = 99) (%)	OR (95% CI)	*P*
Pro-C	85.7	85.7	85.3	1	
Pro-T	3.8	3.6	7.1	1.89 (1.05–3.40)	0.035
Ala-C	3.0	3.1	0.5	0.12 (0.02–0.75)	0.023
Ala-T	7.5	7.5	7.0	0.94 (0.52–1.70)	0.85
Linkage disequilibrium					
D′	0.68	0.67	0.92		
r^2	0.42	0.43	0.42		

OR were adjusted for maternal age and prepregnancy BMI.

associated with the risk of GDM. This could be shown from the haplotype analysis where the combined effect of both polymorphisms was investigated; mothers who carried the haplotypes Ala-C had the lowest risk of GDM, whereas carriers of Pro-T had the highest risk, and the risk in women carrying both variants (haplotype Ala-T) was not modified in comparison with women carrying neither of these variants (haplotypes Pro-C).

This is the first study suggesting a potential role of polymorphisms of the *PPARγ* gene in the etiology of GDM. The association between Pro12Ala, and GDM was not identified in a previous study conducted by Lauenborg *et al.* (21) on 283 cases of GDM compared with 2446 controls. More recently, Pappa *et al.* (22) showed in a case-control study on GDM, that this condition could share many polymorphisms of genes associated with insulin resistance and type 2 diabetes in a Greek population. However, Pro12Ala was investigated and found to be not significantly associated with GDM risk. The OR of GDM associated with carriers of Ala *vs.* Pro/Pro was 0.49, corresponding to a 50% lower risk of GDM in these women. The nonsignificance could clearly be explained by a lack of power in this study of 148 cases and 107 controls. Moreover, our results suggest that the association between the SNP and the disease is recessive; this reduces drastically the power for detecting an effect when it is tested under the dominant model (carriers *vs.* noncarriers) or the codominant model (number of allele carried). In addition, our analysis of haplotypes showed that carrying the Ala allele decreased the risk of GDM significantly only when the C allele of the C1431T SNP was also carried ($P = 0.02$) and not significantly when the T allele of the C1431T SNP was also carried ($P = 0.85$). Studies should be conducted to confirm our results, but it is important that both polymorphisms are investigated simultaneously in such studies.

Doney *et al.* (14) studied the association between the combination of Pro12Ala and C1431T with type 2 diabetes but not with GDM. They did not find exactly the same differences in the distribution of haplotype frequencies in diabetic *vs.* nondiabetic individuals but concluded that the protective effect of the Ala allele was absent when the T allele of C1431T was also carried, which is concordant with our results.

A limitation of our study is that women type 2 diabetes before pregnancy were not included in the EDEN study. This may have selected mothers without genotypes or haplotypes favoring the early onset of type 2 diabetes. However, among women invited to participate, fewer than 1% were not included because of known pregestational diabetes (type 1 or type 2). Furthermore, the Hardy-Weinberg proportions were respected for both polymorphisms and haplotypes in our population; this is not in favor of a strong differential selection bias.

In conclusion, despite the strong linkage disequilibrium between the two polymorphisms Pro12Ala and C1431T of the *PPARγ* gene, the variants of both were associated with GDM in opposite direction, with Ala-C haplotype being protective and Pro-T haplotype a risk factor. Our study suggests for the first time that genetic variations in the *PPARγ* gene could play a role in the susceptibility to develop GDM.

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Address all correspondence and requests for reprints to: Barbara Heude, INSERM U1018 Equipe 10, 16 Avenue Paul

Vaillant Couturier, 94807 Villejuif cedex, France. E-mail: barbara.heude@inserm.fr.

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