Brief Genetics Report

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

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The plasma concentration of the adipocyte-derived peptide adiponectin is decreased in patients with obesity and type 2 diabetes. The adiponectin gene is located on chromosome 3q27, where a diabetes susceptibility locus has been mapped. Adiponectin gene polymorphisms (single nucleotide polymorphisms [SNPs]) have been associated with BMI, insulin sensitivity, and type 2 diabetes in some cross-sectional studies. Our aim was to assess the contribution of these SNPs in the development of features of the insulin resistance syndrome in a 3-year prospective study in ~4,500 French Caucasian subjects from the Epidemiologic Data on the Insulin Resistance Syndrome (DESIR) cohort. For subjects who were normoglycemic at baseline, the 3-year risk of becoming hyperglycemic (diabetes or impaired fasting glucose) was affected by two SNPs: G-11391A and T45G. For G-11391A, the risk was increased in GA carriers (odds ratio [OR] adjusted for sex [versus GG] = 1.60 [95% CI 1.16–2.20]; *P* = 0.004). For T45G, it was increased in GG carriers (OR [versus TT] = 2.71 [1.31–5.60]; *P* = 0.007). After 3 years, GG subjects had a greater increase in BMI (*P* = 0.009) and waist-to-hip ratio (*P* = 0.007). Adiponectin levels at baseline were associated with the development of hyperglycemia (*P* = 0.005), but the predictive effects on the risk for hyperglycemia were independent of adiponectin genotypes. In conclusion, in the DESIR study, variations at the adiponectin locus affect body weight gain, body fat distribution, and onset of hyperglycemia, as well as adiponectin levels. Adiponectin gene SNPs may have several phenotypic effects that co-occur with the development of the metabolic syndrome. *Diabetes* 53:1150–1157, 2004

The role of the newly described adipocyte-derived peptide adiponectin is still poorly understood. Nevertheless, its plasma concentration is decreased in patients with obesity, type 2 diabetes, or coronary artery disease (1,2). The treatment of diabetic animals with adiponectin has been shown (3,4) to improve insulin sensitivity. The adiponectin gene consists of three exons and two introns located on chromosome 3q27, where a diabetes susceptibility locus has been mapped (5,6). Single nucleotide polymorphisms (SNPs) of the adiponectin gene have been associated with BMI, insulin sensitivity, and type 2 diabetes in some cross-sectional studies (7–9), but these associations have not been seen in all studies (10,11). The aim of our study was to assess the effects of these polymorphisms on the 3-year evolution of features of the insulin resistance syndrome in a large Caucasian population of men and women, aged 30–64 years, known as the Epidemiologic Data on the Insulin Resistance Syndrome (DESIR) cohort. The influence of baseline plasma adiponectin levels on the 3-year risk of developing hyperglycemia (impaired fasting glycemia or type 2 diabetes) was also estimated.

RESEARCH DESIGN AND METHODS

The study population consisted of men and women, aged 30–64 years, who participated in DESIR, a 9-year follow-up study that aims to clarify the

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A complete list of DESIR Study Group members can be found in the APPENDIX.

Additional information for this article can be found in two online appendixes at http://diabetes.diabetesjournals.org.

DESIR, Epidemiologic Data on the Insulin Resistance Syndrome; IFG, impaired fasting glucose; SNP, single nucleotide polymorphism; WHR, waist-to-hip ratio.

TABLE 1
Genotype and allele frequencies of adiponectin SNPs in the DESIR cohort

	T0					T3				
Polymorphisms	*n*	Genotypes (%)			Rare allele (%)	*n*	Genotypes (%)			Rare allele (%)
G-11391A	5,186	GG 82.2	GA 16.9	AA 0.9	A 9.4	4,465	GG 82.1	GA 17.0	AA 0.9	A 9.4
C-11377G	5,185	CC 55.2	CG 37.4	GG 7.4	G 26.1	4,464	CC 55.1	CG 37.8	GG 7.1	G 26.0
T45G	5,200	TT 75.3	TG 23.1	GG 1.7	G 13.2	4,479	TT 75.4	TG 23.0	GG 1.6	G 13.1
G276T	5,171	GG 53.8	GT 39.3	TT 6.8	T 26.5	4,454	GG 53.5	GT 39.7	TT 6.8	T 26.6

development of the insulin resistance syndrome (12–14). 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 form. The protocol was approved by the CCPPRB (Comité Consultatif de la Protection des Personnes pour la Recherche Biomédicale) of Bicêtre hospital and the CNIL (Commission Nationale de l'Informatique et des Libertés), and INSERM (Institut National de la Santé et de la Recherche Médicale) was the promoter. At the current time, data are available for two visits, the first at entry (T0) and the second 3 years later (T3). A total of 5,200 subjects were included at T0, and 4,501 had a second examination at T3.

Hyperglycemia (type 2 diabetes or impaired fasting glucose [IFG]) was determined according to the American Diabetes Association criteria (15), in which diabetes is defined as fasting plasma glucose ≥7 mmol/l or treatment by antidiabetic agents and IFG as fasting plasma glucose between 6.1 and 6.9 mmol/l. A total of 3,982 subjects who had both examinations were normoglycemic at T0. Among them, 229 were hyperglycemic at T3 (IFG or type 2 diabetes). These subjects were matched for sex, age, and BMI with 229 subjects who were still normoglycemic at T3.

Weight, height, and waist and hip circumferences were measured by trained personnel, and BMI (kg/m^2) and waist-to-hip ratio were calculated. Venous blood samples were collected in the morning after subjects had fasted for 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 (Kone, Evry, France); total cholesterol, HDL cholesterol, and triglycerides were determined with a DAX24 Technicon or with a KONE; LDL cholesterol was estimated using Dahlen's equation; and fasting serum insulin was measured by an enzymoimmunoassay with IMX (Abbott, Rungis, France) (13).

Fasting plasma adiponectin at baseline (T0) was measured by radioimmunoassay (Linco, St Charles, MO) (sensibility 1 ng/ml, intra- and interassay coefficients of variation 4.4 and 9.9%, respectively) in the 229 subjects who became hyperglycemic and the matched normoglycemic control subjects.

Genotyping. SNP G-11391A and SNP G+276T were genotyped using the fluorogenic 5′ nuclease assay application of the ABI PRISM 7900 HT Sequence Detection System. The conditions for Taqman reaction were as follows: 50°C for 2 min, 95°C for 10 min, and 50 cycles of 95°C for 15 s and 60°C for 1 min.

The C-11377G SNP was genotyped using PCR followed by SNaPshot technique. The SNaPshot primer was specifically designed for each SNP so that it binds to a complementary template one base adjacent to the polymorphism. SNaPshot primers were designed using GenBank accession no. NM004797 for human APM1 mRNA. The conditions for SNaPshot reaction involved denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 30 s. The conditions for SNaPshot reaction were adjusted to the manufacturer's recommendations related to the ABI Prism SNaPshot Multiplex Kit. The completed reaction mixture was then resolved on 96 capillary sequencing machines (Prism 3700; Applied Biosystems). Results were analyzed using Applied Biosystems Genotyper software (version 3.7).

The T+45G SNP was genotyped using a PCR–molecular Beacon technique. The PCR was performed in a 96-well microtitration plate. A total of 200 ng of DNA was amplified in a total volume of 25 μl containing 20 pmol of 5′ and 3′ primers, 0.2 mmol/l dNTPs, 4 mmol/l MgCl2, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 5 pmol of each allele-specific molecular Beacon, and 1 unit of Taq polymerase (Gold Taq; Perkin-Elmer, Paris, France). An initial denaturation and activation of the Taq was performed at 95°C for 10 min, and then the PCR cycling parameters were 20 s at 58°C, 10 s at 72°C, and 10 s at 95°C for 40 cycles in a thermocycler (PTC-200; MJ Research, Watertown, MA). After a final denaturation at 95°C for 2 min, hybridization with the probes was carried out at 60°C for 1 min. The emission of fluorescence was recorded in a plate fluorometer (Fluostar; BMG, Offenburg, Germany) in two wavelength systems: 480–520 nm for fluorescein (FAM) and 520–590 for tetramethylrhodamine (TAMRA). The amplifiers and allele-specific probes were synthetised by Eurogentec (Seraing, Belgium). All of the sequences of primers and probes are available from the authors on request.

Statistical analysis. The association of genotypes with continuous parameters was tested by ANOVA or ANCOVA (multiway ANOVA or ANCOVA for repeated measures, with genotypes, adjusted for sex and other confounding factors). Skewed variables (BMI, triglycerides, insulin, and adiponectin) were log transformed to normalize their distribution before statistical analyses. Differences between glycemic phenotypes for genotype frequencies were compared by χ^2 tests. In the whole population, adjusted odds ratios [ORs] associated with genotypes were calculated by a multivariate logistic regression. To test whether the effect of each SNP was independent of the adiponectin levels effect, a conditional logistic regression analysis was performed in the matched samples (including the subjects in which adiponectin levels were measured). All these statistics were performed by using Systat for Windows software (version 10).

Haplotypes frequencies were estimated by using 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 the two-locus linkage disequilibrium calculator (available from http://www.iop.kcl.ac.uk/IoP/Departments/PsychMed/GEpiBSt/software.shtml).

RESULTS

The genotype and allele frequencies of the baseline population are given in Table 1. For the population with examinations at both baseline and 3 years, the genotype and allele frequencies were almost identical. Genotype distributions were in Hardy-Weinberg equilibrium.

No significant genotype effects on anthropometric (BMI and waist-to-hip ratio [WHR]) and biological phenotypes (plasma lipids, insulin, and glucose levels) could be seen at the time of inclusion (data not shown), except for the T45G SNP. Plasma triglyceride and insulin levels were slightly increased in carriers of the 45G allele ($P = 0.049$ and $P = 0.041$ for triglycerides and insulin, respectively, adjusted for sex, age, and BMI).When looking at interaction effects with time (i.e., the effect of the genotype on the difference between values at 3 years and values at inclusion), there were significant effects of the T45G SNP on BMI ($P = 0.033$) and WHR ($P = 0.010$) (see online appendix Table A1 [available at http://diabetes.diabetesjournals.org]). This was mainly due to the rare GG genotype: for BMI, GG versus TT + TG, $P = 0.009$ (body weight gain in kilograms: 1.04, 1.03, and 2.01 for TT, TG, and GG genotypes, respectively); and for WHR, GG versus TT + TG, $P = 0.007$ (WHR increase: 0.007, 0.010, and 0.023 for TT, TG, and GG genotypes, respectively). These results were similar for both men and women (for BMI change, interaction between sex and genotype, $P = 0.702$; and for WHR, $P = 0.897$). Although changes in body weight and WHR were correlated ($r = 0.210$, $P < 0.001$), the increase in WHR associated with the genotype was still significant

TABLE 2
G-11391A genotype frequencies, according to glycemic status after 3 years in subjects who were normoglycemic at baseline, and ORs (95% CI) for hyperglycemia: the DESIR study

	GG		GA		AA	
Genotype*	*n*	%	*n*	%	*n*	%
Normoglycemic (fasting glucose <6.1 mmol/l)	3,072	82.4	620	16.6	34	0.9
IFG (fasting glucose 6.1–7 mmol/l)	150	75.4	48	24.1	1	0.5
Type 2 diabetes (fasting glucose ≥7 mmol/l or treated for diabetes)	25	80.6	6	19.4	0	0.0
Hyperglycemia (IFG plus type 2 diabetes)	175	76.1	54	23.5	1	0.4
OR (95% CI) for hyperglycemia (adjusted for sex)†	1		1.60 (1.16–2.20), $P = 0.004$		0.55 (0.07–4.04), $P = 0.554$	
			OR A+: 1.55 (1.13–2.12), $P = 0.007$			
OR (95% CI) for hyperglycemia (adjusted for sex, age, BMI, WHR, insulin, glucose, and change in body weight and WHR)†	1		1.65 (1.17–2.32), $P = 0.004$		0.59 (0.08–4.54), $P = 0.613$	
			OR A+: 1.59 (1.13–2.23), $P = 0.008$			

*Genotype frequencies differ according to classes: normoglycemia and hyperglycemia: Pearson $\chi^2 = 7.573$ for df = 2, $P = 0.023$; normoglycemia, impaired fasting glycemia, and diabetes: Pearson $\chi^2 = 8.097$ for df = 4, $P = 0.088$; †by logistic regression (reference = frequent GG genotype).

after adjustment for weight gain ($P = 0.028$). The GA genotype of the G-11391A SNP was associated with a higher increase in glycemia between T0 and T3 ($P = 0.044$).

There was no significant association between genotypes and hyperglycemia (type 2 diabetes or IFG) at the first visit (see online appendix Table A2). However, for subjects normoglycemic at baseline, the risk of developing hyperglycemia was significantly associated with two SNPs, G-11391A and T45G (Tables 2 and 3). Concerning the G-11391A SNP, the sex-adjusted OR of the GA genotype, with reference to the frequent GG, was 1.60 (95% CI 1.16–2.20; $P = 0.004$). When adjusted for multiple factors (sex, age, BMI, WHR, insulinemia, and glycemia, as well as body weight gain and WHR increase), the multiple adjusted OR was 1.65 (1.17–2.32; $P = 0.004$). There was no significant risk associated with the rare homozygous genotype AA due to the small number of individuals carrying this genotype. When pooling all genotypes with the A allele, the sex-adjusted OR of the A carriers was 1.55 (1.13–2.12; $P = 0.007$) and the multiple-adjusted OR was 1.59 (1.13–2.23; $P = 0.008$). Concerning the T45G, the sex-adjusted OR of the GG genotype, with reference to the frequent TT, was 2.71 (1.31–5.60; $P = 0.007$). There was no significant risk associated with the TG genotype (OR 1.13 [0.83–1.55]; $P = 0.448$). Again, when adjusted for multiple factors, the risk associated with GG remained unchanged (OR 2.88 [1.29–6.46]; $P = 0.010$). There were no significant interactions between genotypes and sex, age, BMI, and insulin when tested by the logistic regression.

When using calculated haplotypes instead of single genotypes, neither pairwise combinations (Table 4) nor the full haplotypes containing the variants from the four polymorphisms (data not shown) yielded significant results with respect to the risk of developing hyperglycemia. Nevertheless, *P* values between 0.10 and 0.05 were found for three pairwise combinations (Table 4).

To test whether the prospective effects of genotypes were due to effects on T0 adiponectin levels, we measured plasma adiponectin in the 229 normoglycemic subjects at T0 who became hyperglycemic at T3 and in the 229 subjects matched for sex, age, and BMI. Baseline plasma adiponectin was lower in subjects who later became hyperglycemic than in subjects still normoglycemic,

TABLE 3
T45G genotype frequencies, according to glycemic status after 3 years in subjects who were normoglycemic at baseline, and ORs (95% CI) for hyperglycemia: the DESIR study

	TT		TG		GG	
Genotype*	*n*	%	*n*	%	*n*	%
Normoglycemic (fasting glucose <6.1 mmol/l)	2,816	75.7	847	22.8	56	1.5
IFG (fasting glucose 6.1–7 mmol/l)	140	70.7	50	25.3	8	4.0
Type 2 diabetes (fasting glucose ≥7 mmol/l or treated for diabetes)	24	77.4	6	19.4	1	3.2
Hyperglycemia (IFG plus type 2 diabetes)	164	71.6	56	24.5	9	3.9
OR (95% CI) for hyperglycemia (adjusted for sex)†	1		1.13 (0.83–1.55), $P = 0.448$		2.71 (1.31–5.60), $P = 0.007$	
OR (95% CI) for hyperglycemia (adjusted for sex, age, BMI, WHR, insulin, glucose, and change in body weight and WHR)†	1		1.06 (0.76–1.49), $P = 0.712$		2.88 (1.29–6.46), $P = 0.010$	

*Genotype frequencies differ according to classes: normoglycemia and hyperglycemia: Pearson $\chi^2 = 8.448$ for df = 2, $P = 0.015$; normoglycemia, impaired fasting glycemia, and diabetes: Pearson $\chi^2 = 9.124$ for df = 4, $P = 0.058$; †by logistic regression (reference = frequent and TT genotype).

TABLE 4
Haplotype frequencies according to the prospective risk of hyperglycemia

Haplotype	Hyperglycemic	Normoglycemic	
			$\chi^2 = 6.60$, df = 3,
−11391G-11377C	0.646	0.646	$0.05 < P < 0.10$
−11391G-11377G	0.231	0.261	
−11391A-11377C	0.113	0.091	
−11391A-11377G	0.009	0.001	
			$\chi^2 = 7.12$, df = 3,
−11391G-45T	0.751	0.800	$0.05 < P < 0.10$
−11391G-45G	0.127	0.108	
−11391A-45T	0.087	0.071	
−11391A-45G	0.035	0.022	
			$\chi^2 = 4.94$, df = 3,
−11391G-276G	0.689	0.707	$0.10 < P < 0.25$
−11391G-276T	0.187	0.200	
−11391A-276G	0.042	0.028	
−11391A-276T	0.081	0.064	
			$\chi^2 = 3.28$, 3 df = 3,
−11377C-276G	0.499	0.491	$0.25 < P < 0.50$
−11377C-276T	0.261	0.246	
−11377G-276G	0.235	0.244	
−11377G-276T	0.005	0.019	
			$\chi^2 = 4.78$, 3 df = 3,
−11377C-45T	0.614	0.624	$0.10 < P < 0.25$
−11377C-45G	0.148	0.114	
−11377G-45T	0.224	0.247	
−11377G-45G	0.014	0.016	
			$\chi^2 = 7.38$, df = 3,
45T-276G	0.581	0.608	$0.05 < P < 0.10$
45T-276T	0.256	0.262	
45G-276G	0.150	0.127	
45G-276T	0.013	0.003	

mainly in women (Table 5). Conditional logistic regression showed that the T45G and G-11391A SNPs from one side, and baseline adiponectin levels from the other, were independently associated with the onset of hyperglycemia (Table 6). Moreover, adiponectin levels were significantly higher in −11391A carriers, particularly in women (Table 7). Women carrying the 45G allele also had elevated adiponectin concentrations, but the effect was not statistically significant (Table 7).

TABLE 5
T0 characteristics of subjects in whom adiponectin levels were measured

T3 glycemic status	*n*	Age (years)	BMI (kg/m^2)	Adiponectin (μg/ml)*
Men				
Normoglycemic	149	48.3 ± 8.5	26.6 ± 3.2	22.3 ± 9.8
Hyperglycemic	150	48.2 ± 8.6	26.7 ± 3.6	21.5 ± 9.1
Women				
Normoglycemic	79	50.2 ± 9.7	26.0 ± 3.8	36.2 ± 13.4
Hyperglycemic	79	50.3 ± 9.7	26.3 ± 4.6	31.1 ± 11.9
All				
Normoglycemic	228	49.0 ± 9.0	26.4 ± 3.4	27.1 ± 12.9
Hyperglycemic	229	48.9 ± 9.1	26.5 ± 4.0	24.8 ± 11.1

The adiponectin levels were only measured in T0 normoglycemic people who became hyperglycemic (IFG/type 2 diabetes) at T3 and sex-, age-, and BMI-matched T3 normoglycemic control subjects. *Difference between T3 hyperglycemic and T3 normoglycemic for adiponectin levels: men: $P = 0.451$ (NS); women: $P = 0.015$; all subjects: $P = 0.020$.

DISCUSSION

Our main results in the DESIR cohort show that two adiponectin polymorphisms are associated with the prospective risk of hyperglycemia (IFG or type 2 diabetes) in subjects normoglycemic at baseline. The baseline adiponectin levels also had a predictive value on the risk of developing hyperglycemia in a 3-year period.

The role of adiponectin is far from being completely understood. In particular, in humans, cross-sectional epidemiological studies that associate low levels of adiponectin with obesity, insulin resistance, and coronary heart disease (1,2) do not permit the causality between the lowering of adiponectin and dysregulated metabolism to be established; furthermore, adiponectin may simply be a marker. However, in different mouse models, adiponectin injection lowered hepatic glucose output and increased insulin sensitivity (3). In high-fat, sucrose-gavaged mice, injections of a fragment of adiponectin (gAcrp30) decreased free fatty acids, glucose, and triglyceride postprandial levels by stimulation of β-oxidation (4). Chronic administration prevented a high-fat–induced weight gain even though food consumption was unaffected in treated animals (4). In rhesus monkeys, prospective studies showed that plasma levels of adiponectin decreased at an early phase of obesity and decreased in parallel to the progression of insulin resistance (16). Recent prospective studies show that a low level of adiponectin precedes a decrease in insulin sensitivity (17) and increases the risk of developing type 2 diabetes in Pima Indians (18) and European Caucasians from the EPIC (European Prospective Investigation into Cancer and Nutrition) study (19).

Until now, no prospective data concerning genotypes have been published. Some, but not all, cross-sectional studies are in accordance with our results (summarized in Fig. 1). In this longitudinal study, the GG homozygous genotype of the T45G SNP is associated with increases in body weight and WHR and the occurrence of new cases of hyperglycemia in a 3-year period. These effects are independent of each other, at least in part, which can be linked to the fact that adiponectin can independently influence both glucose and lipid metabolism at a primary level (2). These results are in accordance with Hara et al. (7) who, in a Japanese population, found an OR of 1.70 for type 2 diabetes associated with the GG genotype. Our results are also concordant with results from Stumvoll et al. (8) for BMI. Genome-wide scans have mapped diabetes (6) and metabolic syndrome (5) susceptibility loci in 3q27, where the adiponectin gene is located. It is of interest to note that in the genome scan for the metabolic syndrome, BMI and waist and hip circumferences were among the traits linked to 3q27. Our findings are in contrast with those of Menzaghi et al. (20), who found that the T45-G276 haplotype was at risk for obesity and different features of the insulin syndrome. Actually, the latter study did not show any results for the T45G polymorphism alone, and this could be due to a lack of statistical power, since very few GG homozygous genotypes were included. The −11391 GA genotype is at risk for the onset of hyperglycemia in our study. This result is at contrast with case-control studies in

TABLE 6
Conditional logistic regression parameters (dependent variable = hyperglycemia at T3) in two models, each including adiponectin levels and one polymorphism

n	Factor	OR	95% CI	*P*
228 matched pairs	T0 adiponectin levels	0.60	0.37–0.97	0.038
	45 GG (vs. TT)	4.76	1.02–22.22	0.047
	45 TG (vs. TT)	1.28	0.81–2.01	0.289
225 matched pairs	T0 adiponectin levels	0.56	0.35–0.92	0.022
	−11391 A+ (vs. GG)	1.72	1.06–2.79	0.028

Japanese (7) or in French (9) populations. In the Japanese population, the −11391A allele (numbered −11379 at that time) frequency is very low compared with the French population, which explains the lack of significant effect, although a slight increase in GA genotype could be observed in type 2 diabetes (5.5 vs. 4.4% in control subjects) (7). In the previous French study, the −11391A allele frequency by itself was higher in type 2 diabetes, with a borderline *P* value (0.09), although the −11391G allele was a part of a haplotype at risk for type 2 diabetes (9). A major cause of discrepancy could be the cross-sectional case-control design of these data, as compared with our prospective study in a sample drawn from the general population. As a consequence, subjects from both French studies vary with respect to age (57.6 vs. 47.2 years for ref. 9 and the DESIR, respectively) and BMI (26.8 vs. 24.7 kg/m^2 for ref. 9 and the DESIR, respectively). The phenotype tested for association is also different (type 2 diabetes in ref. 9 versus hyperglycemia [IFG plus type 2 diabetes] in the DESIR). The prospective design of the DESIR allowed us to avoid the biases of case-control studies. Nevertheless, glycemia could be measured only once at follow-up, which introduces a variability for the diagnosis of IFG or type 2 diabetes.

How do these silent SNPs influence these phenotypes? A likely hypothesis is that they could influence adiponectin concentrations. As described in ref. 9, the −11391 SNP in the promoter region is close to a potential regulatory sequence and thus could modulate transcriptional activity. The T45G SNP could have an indirect effect on the adiponectin levels because of its linkage disequilibrium with the −11391 SNP (data not shown). Actually, this hypothesis is not confirmed by our data. Indeed, in the DESIR cohort, the low adiponectin levels at T0 are associated with the prospective risk of hyperglycemia at 3 years of follow-up. This is concordant with the two other prospective studies in Pima Indians (18) and European Caucasians (19). Nevertheless, the analysis by logistic regression demonstrates that the genotypes associated with the risk of hyperglycemia act independently of the T0 adiponectin levels. Moreover, in accordance with Vasseur et al. (9), these at-risk genotypes increase, more or less, baseline adiponectin levels.

With regard to this apparent contradiction, sexual differences might be of importance. In DESIR, the genotype effects on the prospective risk of hyperglycemia are very similar between men and women, without a sex-by-genotype interaction. On the other hand, baseline adiponectin levels are increased with at-risk genotypes mainly in women. These levels are protective mostly in women, too. In the EPIC study (19), the prospective effect of adiponectin levels is also slightly higher in women. Thus, to summarize, adiponectin levels vary significantly with sex, but prospective genotype effects are seen in both sexes.

Mechanisms other than an effect on adiponectin concentration could be involved. The ability to multimerize can play a role in adiponectin action. This ability is modulated by sex (21,22), different metabolic challenges (22), and some rare mutations of adiponectin (22). Since both SNPs associated with the risk do not modify the protein structure, they might be in disequilibrium with mutations of this kind.

Although the G-11391A and T45G SNPs are in positive linkage disequilibrium, both independently influence the studied phenotypes. The standardized disequilibrium between both SNPs is significant but weak (D′ = 0.120 in

TABLE 7
T0 adiponectin concentration (μg/ml) according to adiponectin gene polymorphisms

	Genotypes			
	GG	GA	AA	*P**
G-11391A				
Men	21.4 ± 9.7 (239)	23.7 ± 8.3 (57)	31.9 (1)	0.020
Women	31.4 ± 12.1 (121)	41.3 ± 13.0 (35)	—	<0.001
C-11377G	CC	CG	GG	
Men	22.5 ± 9.3 (180)	20.5 ± 9.2 (97)	23.7 ± 11.0 (21)	0.112
Women	32.2 ± 13.0 (81)	35.8 ± 12.5 (67)	30.1 ± 13.9 (9)	0.102
T45G	TT	TG	GG	
Men	21.5 ± 9.4 (219)	23.5 ± 9.7 (73)	19.1 ± 7.4 (7)	0.208
Women	32.8 ± 11.8 (121)	35.4 ± 15.9 (33)	44.4 ± 16.2 (4)	0.300
G276T	GG	GT	TT	
Men	22.2 ± 10.2 (149)	21.7 ± 8.7 (125)	21.7 ± 8.7 (21)	0.997
Women	33.8 ± 12.8 (93)	33.8 ± 13.2 (53)	31.3 ± 13.5 (11)	0.712

Data are means ± SD (*n*). The adiponectin levels were only measured in future hyperglycemic (IFG/type 2 diabetes) and sex-, age-, BMI-matched normoglycemic control subjects. *GG versus A+.

	Phenotype	Japanese population (7) 480 non diabetic, 384 T2D	Japanese population (23) 183 non diabetic, 164 T2D, all non obese	French population (9) 1373 non diabetic, 743 T2D	Italian + American population (20) 304 + 413 non diabetic, 310 T2D	German population (8) 371 non diabetic subjects	French population (This study) 5200 subjects from the general population with a 3 year follow up
G-11391A	T2D	=	NT	A ↗ borderline ($P = 0.09$)	NT	NT	A ↗ incidence (T2D+IFG)
	Adiponectin levels	=	NT	A ↗	NT	NT	A ↗
C-11377G	T2D	C borderline ↗ ($P = 0.10$)	C ↗	G ↗	NT	NT	=
	Adiponectin levels	=	NT	G ↘	NT	NT	=
T45G	T2D	G ↗	=	=	=	G ↘ insulin sensitivity	GG ↗ incidence (T2D+IFG)
	Adiponectin levels	=	NT	G ↗	NT	NT	=
	BMI	=	NT	NT	=	G ↗	GG ↗ weight gain
G276T	T2D	G ↗	G borderline ↗ (P=0.08)	=	G ↗ insulin resistance but not T2D	NT	=
	Adiponectin levels	GG ↘ when BMI>26.7	NT	T ↗	NT	NT	=

* =: no association, ↘: negative association, ↗: positive association, NT: not tested

FIG. 1. Summary of association studies on adiponectin SNPs. *All studies except this one were cross-sectional.

DESIR). When tested in the same logistic regression equation, both genotype effects on the risk of hyperglycemia remain significant. Moreover, when testing the T45G polymorphism after removal of the −11391A carriers from the sample (data not shown), the association persists.

To our knowledge, this is the first study providing results on adiponectin gene polymorphisms from longitudinal data. Our study demonstrates a predictive effect of a variation at the gene level of adiponectin on body weight gain, body fat distribution, and the onset of hyperglycemia and confirms the predictive value of low adiponectin levels on the risk of hyperglycemia. This is in favor of a causal role for adiponectin on these metabolic features in humans.

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APPENDIX

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