Genetics of the *APM1* Locus and Its Contribution to Type 2 Diabetes Susceptibility in French Caucasians

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We have carried out a detailed reexamination of the genetics of the *APM1* locus and its contribution to the genetic basis of type 2 diabetes susceptibility in the French Caucasian population. The G allele of single nucleotide polymorphism −11426 in the *APM1* promoter showed modest association with type 2 diabetes (odds ratio 1.44 [95% CI 1.04–1.98]; $P = 0.03$), providing corroborative evidence that single nucleotide polymorphisms in the *APM1* promoter region contribute to the genetic risk of type 2 diabetes. A "sliding window" analysis identified haplotypes 1-1-1, 1-1-1-1, and 1-1-1-1-1 as being strongly protective against type 2 diabetes ($P \leq 0.0001$). Evidence is presented that the *APM1* gene is a locus of low linkage disequilibrium, high haplotype diversity, and high recombination. We were unable to obtain data to support the hypothesis that genetic variation in the *APM1* gene is a major contributor to the type 2 diabetes linkage result at chromosome 3q27. Finally, in families with early-onset type 2 diabetes, we obtained suggestive evidence of a linkage peak for serum adiponectin levels (logarithm of odds = 2.1) that closely matched the position of the type 2 diabetes linkage peak. This result indicated that the type 2 diabetes susceptibility locus at 3q27 influences both genetic predisposition to type 2 diabetes and serum adiponectin levels in patients with type 2 diabetes. *Diabetes* 53:2977–2983, 2004

The strong correlation between the twin global epidemics of obesity and type 2 diabetes and the identification of obesity as a major cause of type 2 diabetes is now well established (1–4). It has also become clear that adipose tissue rather than being a passive energy storehouse is, in fact, an active endocrine organ secreting a variety of proteins that regulate glucose levels, lipid metabolism, and energy homeostasis (5–7), including leptin (8,9), tumor necrosis factor-α (10,11), and resistin (12,13). Another adipose-secreted protein that has attracted a lot of attention recently is adiponectin, an abundant plasma protein encoded by the *APM1* gene. In pharmacological studies, the administration of adiponectin caused weight loss and lowered plasma glucose in mice that were fed a high-fat and -sucrose diet (14). Adiponectin was also shown to stimulate glucose uptake and fatty acid oxidation via the phosphorylation and activation of 5′-AMP–activated protein kinase (15). The data from adiponectin knockout studies have been conflicting, with adiponectin-deficient mice exhibiting moderate insulin resistance with glucose intolerance (16); insulin resistance, but only when fed a high-fat and -sucrose diet (17); and normal plasma glucose and insulin levels (18). An interesting prospective study in rhesus monkeys showed that adiponectin levels decreased in parallel with the development of insulin resistance and type 2 diabetes (19–21). In humans, adiponectin levels are significantly reduced in individuals with obesity (22) and type 2 diabetes (19) relative to control subjects and are correlated with the level of insulin sensitivity and insulinemia (21). These observations suggested that low plasma adiponectin may contribute to the pathogenesis of insulin resistance and type 2 diabetes and, therefore, that the *APM1* gene is a candidate type 2 diabetes susceptibility gene.

The *APM1* gene maps to chromosome 3q27, a region identified as a susceptibility locus for the metabolic syndrome (23) and type 2 diabetes (24). Scanning the gene for single nucleotide polymorphisms (SNPs) in the Japanese (25) and French Caucasian (26) populations revealed 10 common (frequency >5%) SNPs. SNPs +45 and +276, in exon 2 and intron 2, respectively, were found to be associated with type 2 diabetes in the Japanese (25), whereas in French Caucasians, a haplotype including SNPs −11391 and −11377 in the proximal promoter region of the *APM1* gene was associated with both adiponectin levels and type 2 diabetes (26). In a recent study in the Swedish Caucasian population (27), SNPs −11426 and −11377 were associated with type 2 diabetes, providing additional evidence that SNPs in the promoter region of the *APM1* gene contribute to the genetic risk of type 2 diabetes.

In this report, we present a detailed reexamination of the genetics of the *APM1* locus and its contribution to the genetic basis of susceptibility of type 2 diabetes in the French Caucasian population. Genetic variants in the *APM1* gene were tested for association with type 2 diabetes in a new case-control study. We characterized the relationship between the pattern of linkage disequilibrium (LD) across the *APM1* gene, the *APM1* haplotype diversity, and the *APM1* locus-specific recombination rate. In addition, we assessed the contribution of *APM1* SNPs to the evidence for linkage of type 2 diabetes to chromosome 3q27 (24). Finally, evidence is presented that the type 2

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AEL, association with the evidence for linkage; ASP, affected sibling pair; EM, expectation maximization; HTR, Haplotype Trend Regression; ibd, identical by descent; LD, linkage disequilibrium; LOD, logarithm of odds; SNP, single nucleotide polymorphism.

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diabetes susceptibility locus at 3q27 influences serum adiponectin levels in individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS

The American Diabetes Association 2003 criteria (28) for the classification of diabetes or normoglycemia were applied. The subset of 55 families with early-onset type 2 diabetes (age at diagnosis <46 years) who produced the linkage result at chromosome 3q27 was described previously (24). These families had the following affected sibling pair (ASP) composition: 41 families had one ASP, 11 had three ASPs, and 3 had six ASPs, giving a total of 92 ASPs. Our case-control study was carried out with a cohort of 812 unrelated type 2 diabetic subjects (age 57 ± 11 years; BMI 34.2 ± 10.3 kg/m^2; male/female 48:52%) and 1,044 unrelated normoglycemic subjects (age 49 ± 14 years; BMI 30.7 ± 10.4 kg/m^2; male/female 34:66%). The type 2 diabetic patients were composed of 307 probands from type 2 diabetic families, 274 probands from obese families (29), and 231 singleton type 2 diabetic subjects who were recruited from the Corbeil region of France. The normoglycemic control subjects were composed of 264 husbands or wives from type 2 diabetic families, 268 subjects from a general population recruited in the Fleurbaix region of France, and 512 obese family probands. Case patients and control subjects were of Northern French Caucasian ancestry. Informed consent was obtained from all subjects, and the study was approved by the local ethics committees.

Genotyping. *APM1* SNP genotypes were generated by direct sequencing of PCR products or by the LightCycler technique (Roche Diagnostics, Mannheim, Germany). Our genotyping focused on the SNPs that had previously given positive associations with type 2 diabetes. Thus, SNPs −3971 and +712 were genotyped in less than half of the case-control cohort.

Haplotype analyses. Two different methods for haplotype analysis were used, each of which provided different and complementary data. The program PHASE (30,31) implements a Bayesian statistical method for reconstructing haplotypes from genotype data. In simulation experiments, the mean error rate with PHASE was approximately half that obtained with the expectation maximization (EM) algorithm. The current version (2.0.2) outputs an estimate of the local population recombination rate parameter, ρ. The Haplotype Trend Regression (HTR) program (32) uses the EM algorithm to infer haplotypes. A key facility of this program is its ability to test the inferred haplotype probabilities for association with the phenotype (qualitative or quantitative) of interest using a regression-based approach. Empirical *P* values were derived by permutation using 10,000 replicates. HTR also provides a "sliding window" mode of haplotype analysis to test for associations with haplotypes in a particular region of the gene, such as in the promoter. However, this approach raises the nettlesome problem of multiple testing. For each window size of x SNPs, the actual number of windows was given by $(8 - x) + 1$, amounting to a grand total for eight SNPs of 35 windows. If we then multiply 35 by the average number of common haplotypes in each window ($n = 5$), we get a conservative Bonferroni correction factor of 175. In an attempt to strike a balance between maximizing the statistical power for detecting haplotype associations and minimizing the level of missing data, subjects with a minimum of six available SNP genotypes (of eight) were included for haplotype estimation. A total of 713 case patients and 624 control subjects met this inclusion criterion.

Statistical analyses. Comparisons of SNP allele and PHASE haplotype frequencies in case patients and control subjects were performed with the χ^2 test. Testing SNP alleles for association with quantitative traits was carried out with the Wilcoxon-Kruskal-Wallis test (30–32). Pairwise LD was calculated with the GOLD software package (33) from the haplotype counts output by PHASE. Serum adiponectin levels were measured as described previously (25) and corrected for sex, age, and BMI (26). Linkage analyses were carried out using the Genehunter software package (34). Type 2 diabetes logarithm of odds (LOD) scores were calculated using the MLS statistic (35) with all ASPs included but weighted by 2/n affected siblings. LOD scores for adiponectin levels were carried out using the EM Haseman-Elston regression statistic (36) with only the first ASP from each sibship included in the analysis (34,36). The information content for all microsatellite markers in the 3q27-qter region exceeded 0.85 (24).

Association with the evidence for linkage. For each SNP, we tested both alleles for association with the evidence for linkage (AEL). Following a modified version of the method of Horikawa et al. (37), we postulated that if allele 1 (the major allele) was the predisposing allele, then ASPs that contribute to the linkage would have concordant 1x-1x genotypes, i.e., 1/1-1/1 or 1/2-1/2 genotypes. Similarly, if allele 2 (the minor allele) were the risk allele, then ASPs would have concordant x2-x2 genotypes, i.e., 1/2-1/2 or 2/2-2/2 genotypes. This is equivalent to a dominant model of inheritance. Accordingly, a set of ASPs with concordant genotypes 1/1-1/1 or 1/2-1/2 and a set of ASPs with concordant genotypes 1/2-1/2 or 2/2-2/2 were identified. Expected identical-by-descent (ibd) allele-sharing proportions under the null hypothesis of no AEL were calculated for each concordant ASP set conditional on allele frequency, concordant genotype, and the estimated ibd sharing distribution for peak linkage marker D3S1580 (24). The probability of an ASP having the concordant genotype 1/1 or 2/2 is given by p^{4-s} or q^{4-s}, respectively, where p and q are the frequencies of alleles 1 and 2 and s is the number of alleles shared ibd. The probability of an ASP having the concordant genotype 1/2 given 0, 1, and 2 alleles shared ibd is $(2pq)^2$, pq, and 2pq, respectively. Observed ibd sharing proportions were determined by counting the number of concordant ASPs in each ibd class, as indicated by the estimated ibd sharing status of the ASP. An observed allele-sharing proportion that is higher than the null level presents evidence of AEL. The statistical significance of the difference between the observed and expected allele sharing was determined by gene-dropping simulation, preserving the ibd status of ASPs, as described previously (29,37). Empirical *P* values were all >0.05 with 100 simulations.

RESULTS

We have performed a case-control genetic association study of *APM1* genetic variants in the French Caucasian population, with the aim of replicating the results of a previous report indicating that SNPs in the *APM1* promoter region are associated with type 2 diabetes (26). The genotype distribution was in accordance with Hardy-Weinberg equilibrium for all SNPs (data not shown). There were no significant differences in SNP allele or haplotype frequencies between men and women, and no additional associations were uncovered by stratifying for sex (data not shown).

Of the 10 common SNPs in the *APM1* gene (25,26), SNP pairs +45 and +349 and +712 and +2019 are in complete LD ($d^2 = 1$) (26), and so data are presented only for the eight nonredundant SNPs shown in Table 1. The G allele of SNP −11426 in the *APM1* promoter was the only variant nominally associated with type 2 diabetes (odds ratio [OR] 1.44 [CI 1.04–1.98]; $P = 0.03$), a result that was obtained with 551 case patients and 551 control subjects. We did not find evidence for association of SNP −11426 with the homeostasis model assessment of insulin resistance index (38) or with adiponectin levels in either type 2 diabetic patients or normoglycemic control subjects (data not shown).

In haplotype analyses, an important factor to consider is that of missing genotype data, which has a detrimental effect on the accuracy of inferred genotypes. As shown in Table 1, the degree of missing genotype data varied across the eight SNPs analyzed. Thus, in an attempt to strike a balance between maximizing the statistical power for detecting haplotype associations and minimizing the level of missing data, subjects with a minimum of six available SNP genotypes (of a total of eight) were included for haplotype estimation. The haplotype frequency distribution of case patients and control subjects inferred by PHASE is shown in Table 2. The average posterior probability of a haplotype pair across the entire dataset was moderate (0.68 ± 0.23). The most common haplotype spanning the *APM1* gene (1-1-1-1-1-1-1-1) was associated with normoglycemia, both with a simple χ^2 analysis of the haplotype frequencies output by PHASE (OR 0.78 [95% CI 0.64–0.96]; $P = 0.017$) and with the HTR software ($P = 0.008$). A "sliding window" analysis of haplotype associations was also carried out with the HTR software. The haplotypes 1-1-1, 1-1-1-1, and 1-1-1-1-1 all gave empirical *P* values ≤0.0001, indicating strong association with normoglycemia (Table 3). These results were still significant

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TABLE 1
APM1 SNP allele frequencies in type 2 diabetic patients and normoglycemic control subjects

SNP (dbSNP ID)	*n*	Allele 1	Allele 2	*P*	OR (95% CI)
−11426 (NA)		A	G		
Type 2 diabetic	551	1,007 (0.91)	95 (0.09)	**0.03**	**1.44 (1.04–1.98)**
Normoglycemic	551	1,034 (0.94)	68 (0.06)		
−11391 (NA)		G	A		
Type 2 diabetic	775	1,388 (0.90)	162 (0.10)	0.09	
Normoglycemic	944	1,723 (0.91)	165 (0.09)		
−11377 (rs266729)		C	G		
Type 2 diabetic	812	1,191 (0.73)	433 (0.27)	0.75	
Normoglycemic	1,044	1,541 (0.74)	547 (0.26)		
−4041 (rs822395)		A	C		
Type 2 diabetic	762	979 (0.64)	545 (0.36)	0.15	
Normoglycemic	894	1,191 (0.67)	597 (0.33)		
−3971 (rs822396)		A	G		
Type 2 diabetic	558	921 (0.83)	195 (0.17)	0.19	
Normoglycemic	432	732 (0.85)	132 (0.15)		
+45 (rs2241766)		T	G		
Type 2 diabetic	740	1,274 (0.86)	206 (0.14)	0.52	
Normoglycemic	915	1,561 (0.85)	269 (0.15)		
+276 (rs1501299)		G	T		
Type 2 diabetic	701	1,027 (0.73)	375 (0.27)	0.17	
Normoglycemic	893	1,269 (0.71)	517 (0.29)		
+712 (rs3774261)		G	A		
Type 2 diabetic	346	396 (0.57)	296 (0.43)	0.25	
Normoglycemic	324	391 (0.60)	257 (0.40)		

Genotype data were generated from a cohort of 812 type 2 diabetic patients and 1,044 normoglycemic control subjects of French Caucasian ancestry. *P* value <0.05 with associated OR shown in bold. NA, no ID number available.

after a conservative Bonferroni correction for the number of tests carried out in the sliding window analyses. We did not find evidence for association of any haplotype with the homeostasis model assessment of insulin resistance index (38) or with adiponectin levels in either type 2 diabetic patients or normoglycemic control subjects (data not shown). As a group, the pooled rare haplotypes were weakly associated with type 2 diabetes by χ^2 analysis (OR 1.19 [95% CI 1.01–1.40]; $P = 0.03$). The frequency of the "1-2" promoter haplotype previously reported to be associated with type 2 diabetes (26) was higher in type 2 diabetic patients than in normoglycemic control subjects (27.1 vs. 24.4%), but the difference was not statistically significant in this study ($P = 0.11$).

We compared the extent of pairwise LD across the set of eight nonredundant *APM1* SNPs, as indicated by the metrics D′ and d^2. The median of all D′ values was 0.89, indicating high LD, as reported previously (26), but the median d^2 value was only 0.03, indicating a low level of LD across the *APM1* gene (Table 4). D′ is scaled to be independent of allele frequency and therefore is typically higher than d^2 (39), but even so, the level of LD indicated by the two metrics seemed contradictory. To investigate this further, we reexamined the haplotype structure of the *APM1* gene. Table 2 shows that six common (frequency ≥5%) haplotypes spanning the gene were identified, accounting for 68% of control chromosomes. This is indicative of a higher level of haplotype diversity than that reported in the majority of previous candidate gene studies, where three to six common haplotypes accounted for 80–90% of chromosomes (40–42). From the *APM1* haplotype data, the population recombination rate parameter ρ

TABLE 2
APM1 haplotype distribution in case and control subjects

Haplotype	Type 2 diabetes (*n* = 713)	Normoglycemia (*n* = 624)
11111111	15.7	19.2
11211111	13.7	13.4
11111122	11.4	12.7
11122111	10.7	9.4
11111212	7.0	8.1
12121122	5.7	5.4
Pooled rare	35.7	31.8
Total	100	100

Data are %. Subjects with a minimum of six available SNP genotypes (out of a maximum of eight) were included for haplotype estimation. Haplotype frequencies were estimated with PHASE (30,31). Haplotypes with a frequency <5% were defined as rare and pooled together.

TABLE 3
APM1 haplotypes associated with normoglycemia in a "sliding window" analysis

SNP window size	Haplotype	HTR *P*	χ^2 *P*
2	11xxxxxx	0.015	0.065
3	111xxxxx	0.0001	0.006
4	1111xxxx	0.0001	0.002
5	11111xxx	<0.0001	0.003
6	111111xx	0.006	0.018
7	x1111111	0.009	0.029
8	11111111	0.008	0.017

Estimation of APM1 haplotypes and their association with type 2 diabetes or normoglycemia were carried out with the HTR software package (32). For each SNP window size, the haplotype with the strongest association with normoglycemia is shown. The empirical *P* values output by HTR are shown next to *P* values from a χ^2 analysis of the relevant haplotype frequencies output by PHASE. x, any allele.

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TABLE 4
Pairwise LD values for SNPs spanning the APM1 gene

		D′							
		−11426	−11391	−11377	−4041	−3971	+45	+276	+712
	−11426		1.00	1.00	0.92	0.89	0.91	0.29	0.25
	−11391	0.01		1.00	0.94	1.00	0.08	0.62	0.92
	−11377	0.02	0.04		0.13*	0.14*	0.47	0.83	0.70
d^2	−4041	0.03	0.19	0.00*		0.96	0.37	0.00*	0.15
	−3971	0.01	0.02	0.00*	0.41		0.94	0.86	0.88
	+45	0.01	0.00	0.01	0.01	0.03		0.99	0.91
	+276	0.01	0.12	0.09	0.00*	0.06	0.06		0.95
	+712	0.01	0.13	0.12	0.01	0.12	0.20	0.46	

Pairwise LD was calculated with the GOLD software package (33) from the combined haplotype counts of 713 type 2 diabetic patients and 624 control subjects output by PHASE (30,31). *P* values were all <0.001 except for those marked with *, representing SNP pairs −11377 and −4041 ($P = 0.006$), −11377 and −3971 ($P = 0.04$), and −4041 and +276 ($P = 0.89$).

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was estimated by PHASE to be 0.092, >2 orders of magnitude higher than the average value of 0.0004 (43), indicating that the *APM1* gene is a locus of high recombination and, therefore, of low LD. We tested the *APM1* SNPs for AEL of type 2 diabetes to 3q27, using a modified version of the linkage partitioning method described by Horikawa et al. (37). Briefly, this involved calculating the expected and observed allele-sharing proportions for ASPs with concordant genotypes, conditional on the estimated allele-sharing distribution of the original genome scan dataset (24). The results, shown in Table 5, indicate that under the null hypothesis of no AEL, every one of the *APM1* SNP alleles would be expected to exhibit an allele-sharing proportion that is higher than the original linkage result. This reflects the inherent bias toward linkage associated with ASP subsets with concordant genotypes. It is clear that none of the *APM1* SNP alleles exhibited an allele-sharing proportion that was higher than the null value. Each of the common haplotypes and the pooled rare *APM1* haplotypes also did not exhibit an allele-sharing proportion that was higher than the null value (data not shown). Because the number of ASPs in these analyses was relatively small, we confirmed these results by a simulation-based approach (29,37) (data not shown). Thus, the data argued against the hypothesis that genetic variation in the *APM1* gene is associated with the evidence for linkage.

Because serum adiponectin levels are significantly reduced in individuals with type 2 diabetes (19–21), we postulated that the type 2 diabetes locus at 3q27 may modulate adiponectin levels in the affected members of the families who produced the type 2 diabetes linkage result. Serum adiponectin levels were measured in these families, and linkage analysis was carried out with adiponectin levels as a quantitative trait using the EM Haseman-Elston regression method, as implemented in Genehunter. The other quantitative trait linkage analysis methods packaged in Genehunter (the maximum likelihood QTL variance and the nonparametric QTL methods) (34) gave very similar results (data not shown). Figure 1 shows that we identified evidence suggestive of a linkage peak for serum adiponectin levels, with a LOD score of 2.1, that closely matched the position of the type 2 diabetes linkage peak. This result was consistent with the idea that the type 2 diabetes susceptibility locus at 3q27 influences both a genetic predisposition to type 2 diabetes and serum adiponectin levels.

DISCUSSION

The aim of the present study was to carry out a detailed reexamination of the genetics of the *APM1* locus and its contribution to the genetic basis of susceptibility to type 2 diabetes in the French Caucasian population. The G allele of SNP −11426 exhibited modest association with type 2 diabetes, in agreement with a recent study in the Swedish population (27), providing corroborative evidence that SNPs in the promoter region of the *APM1* gene contribute to the genetic risk of type 2 diabetes. In the context of study design, it is important to note that the control subjects were younger than the case patients. This will tend to decrease the power to detect associations with type 2 diabetes, since it can be postulated that a small proportion of the control subjects are latent type 2 diabetic patients who will go on to develop type 2 diabetes at a later age. We obtained strong evidence that haplotypes that contain the major *APM1* SNP alleles are associated with normoglycemia, indicating that these haplotypes have a protective effect against type 2 diabetes. The implication of the associations reported in the present study is that these variants affect *APM1* gene expression and, ultimately thereby, plasma adiponectin levels. However, we were unable to detect evidence that SNP −11426 or the "1" haplotypes were associated with adiponectin levels. Although this seems to weaken the case for these associations, we note the results of two genome scans for adiponectin levels, in the Northern European (44) and Pima Indian populations (45), both of which indicated that the *APM1* gene has only a minor influence on circulating adiponectin levels. Thus, if these variants do not influence circulating adiponectin levels, then an alternative hypothesis is that they have a local autocrine/paracrine effect on adiponectin levels in adipose tissue. Further studies are warranted to directly assess the effect of these variants on *APM1* gene expression and the mechanism(s) by which they modulate type 2 diabetes susceptibility.

The average posterior probability of haplotype inference across the dataset was moderate; therefore, we have to interpret the haplotype associations with type 2 diabetes and normoglycemia with caution. However, we note that the haplotype distribution in this study is very similar to

TABLE 5
AEL analysis: APM1 SNPs

SNP	Allele		z_0	z_1	z_2	zL	No. of ASPs
Original linkage result			0.07	0.49	0.44	0.68	92
−11426	1						
		Exp	0.06	0.47	0.47	0.71	85
		Obs	0.06	0.50	0.44	0.69	78
	2						
		Exp	0.01	0.36	0.67	0.85	7
		Obs	0.00	0.62	0.38	0.69	13
−11391	1						
		Exp	0.06	0.46	0.49	0.72	82
		Obs	0.09	0.52	0.39	0.65	77
	2						
		Exp	0.02	0.34	0.63	0.80	11
		Obs	0.00	0.44	0.56	0.78	9
−11377	1						
		Exp	0.05	0.40	0.56	0.76	67
		Obs	0.06	0.45	0.48	0.71	62
	2						
		Exp	0.04	0.32	0.63	0.79	29
		Obs	0.07	0.41	0.52	0.72	27
−4041	1						
		Exp	0.04	0.38	0.58	0.77	62
		Obs	0.08	0.36	0.57	0.75	53
	2						
		Exp	0.04	0.33	0.64	0.80	35
		Obs	0.06	0.33	0.61	0.78	36
−3971	1						
		Exp	0.05	0.43	0.51	0.73	77
		Obs	0.10	0.46	0.44	0.67	72
	2						
		Exp	0.02	0.33	0.62	0.79	18
		Obs	0.13	0.39	0.48	0.67	23
+45	1						
		Exp	0.05	0.43	0.51	0.73	77
		Obs	0.10	0.49	0.41	0.66	73
	2						
		Exp	0.02	0.33	0.62	0.79	18
		Obs	0.08	0.42	0.50	0.71	12
+276	1						
		Exp	0.04	0.39	0.57	0.76	65
		Obs	0.03	0.52	0.45	0.71	58
	2						
		Exp	0.04	0.32	0.63	0.79	32
		Obs	0.00	0.56	0.44	0.72	18
+712	1						
		Exp	0.04	0.36	0.60	0.77	57
		Obs	0.02	0.43	0.55	0.76	44
	2						
		Exp	0.04	0.33	0.63	0.80	41
		Obs	0.00	0.50	0.50	0.75	28

The estimated allele-sharing proportions expected (Exp) under the null hypothesis of no AEL are compared with the observed (Obs) allele-sharing proportions for *1*) ASPs with concordant 1/1-1/1 and 1/2-1/2 genotypes (representing the AEL data for allele 1 [the major allele]) and *2*) ASPs with concordant 1/2-1/2 and 2/2-2/2 genotypes (representing the AEL data for allele 2). zi, the proportion of ASPs sharing i alleles ibd; zL, the overall allele-sharing proportion = (z1 + 2z2)/2. The estimated allele sharing distribution for peak linkage marker D3S1580 in the original dataset (24) is shown.

that reported previously (26) and, moreover, that the major alleles of SNPs −11426 to −3971 all have a higher frequency in control subjects (Table 1), consistent with the "1" haplotype association with normoglycemia. In general, the case-control data presented here tend to support the hypothesis that genetic variation in the *APM1* gene modulates the risk of type 2 diabetes in Northern Europeans. However, an independent large-scale case-control study that contains thousands of subjects is urgently required for definitive confirmation of the role of *APM1* variants in the genetic basis of type 2 diabetes susceptibility.

We have presented evidence that the *APM1* gene is an example of a locus with a higher-than-average recombination rate and, therefore, high haplotype diversity and low LD. A recent survey of haplotype diversity across 100 candidate genes reported that an average of 4.5 common haplotypes represented 75% of chromosomes in individuals of European descent (46). This compares with six haplotypes representing 68% of chromosomes for the *APM1* gene, consistent with the idea that the *APM1* gene has a higher-than-average haplotype diversity.

The *APM1* gene is a strong type 2 diabetes candidate gene in a region of linkage to type 2 diabetes, and it was important to document the results of testing *APM1* SNPs for AEL (45). We have demonstrated that none of the *APM1* SNPs exhibited a level of allele sharing that was higher than that expected under the null hypothesis of no AEL. Thus, the data argue against the hypothesis that *APM1* SNPs are strongly associated with the evidence for linkage at 3q27 and indicate that another, as-yet-unidentified, gene or genes are responsible for the linkage at 3q27.

In the affected members of the early-onset families who produced the type 2 diabetes linkage result at 3q27, we identified evidence suggestive of a linkage peak for serum adiponectin levels that closely matched the position of the type 2 diabetes linkage peak. This result suggested the possibility that the type 2 diabetes susceptibility locus at 3q27 influences serum adiponectin levels and is consistent with the observation that serum adiponectin levels are significantly reduced in individuals with type 2 diabetes, independent of the level of adiposity (19–21). The adiponectin linkage result was obtained despite the fact that most of the type 2 diabetic subjects in the genome scan families are receiving insulin-sensitizing drugs (24) that would be expected to have an effect on adiponectin levels, through the close correlation between insulin sensitivity and adiponectin levels (20,21). We can only speculate that if adiponectin levels had been measured before the onset of pharmacological intervention, then the linkage evidence may have been stronger. The 3q27 type 2 diabetes susceptibility locus may influence serum adiponectin levels either directly, by affecting *APM1* gene expression, or indirectly, by affecting insulin sensitivity. Work is currently in progress to identify the type 2 diabetes susceptibility gene(s) at 3q27 that explains the linkage and characterize the mechanism(s) by which it modulates serum adiponectin levels.

In conclusion, type 2 diabetes is a complex trait that depends on the interaction of genetic and environmental factors. The "obesogenic" environment that exists in most developed countries acts to increase adipose mass, which has the effect of decreasing adiponectin levels and insulin sensitivity. The results presented here suggest the testable hypothesis that *APM1* variants interact with the environment to influence type 2 diabetes susceptibility via an

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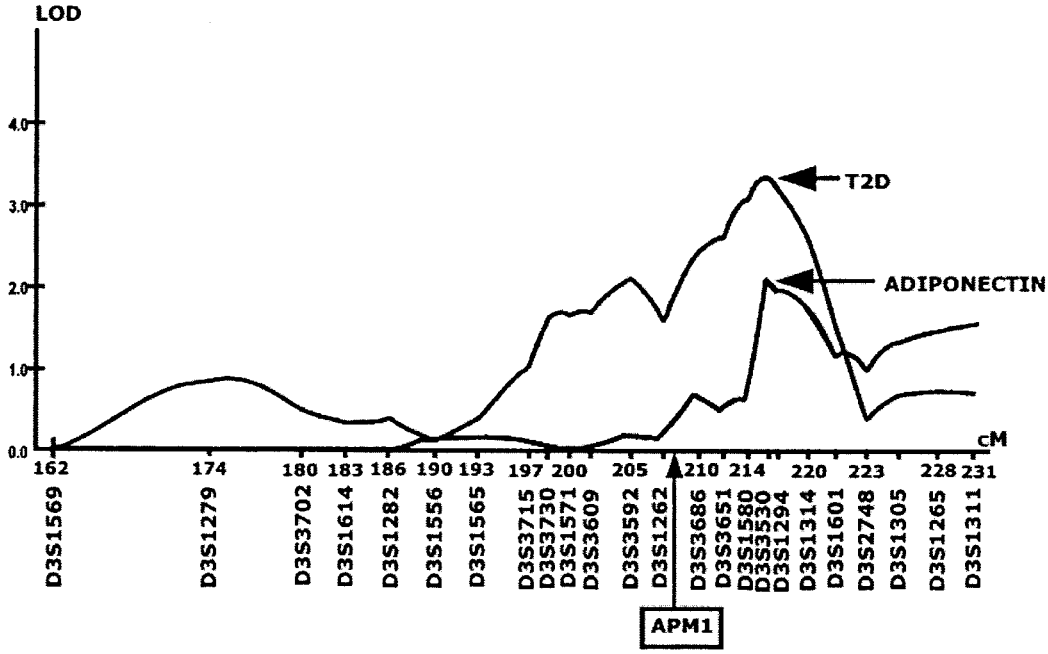

FIG. 1. Linkages of type 2 diabetes and serum adiponectin levels to chromosome 3q27 in French early-onset diabetic families. The approximate position of the *APM1* gene relative to the two linkage peaks is shown.

effect not on circulating adiponectin levels but rather on the adiponectin level within adipose tissue. Thus, the G allele of SNP −11426 predisposes to type 2 diabetes because it exacerbates the obesogenic effect of the environment by promoting low adiponectin levels and a low insulin sensitivity in adipose tissue, whereas the "1" haplotype is protective against type 2 diabetes because it counters the environment by favoring high adiponectin levels and a high insulin sensitivity in adipose tissue.

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