Polymorphism of the 3′-Untranslated Region of the Leptin Receptor Gene, but Not the Adiponectin *SNP45* Polymorphism, Predicts Type 2 Diabetes

A population-based study

Monica Nannipieri, md[1]
Rosalinda Posadas, phd[1]
Alessandra Bonotti, bm[1]
Ken Williams[2]

Clicerio Gonzalez-Villalpando, md[3]
Michael P. Stern, md[2]
Ele Ferrannini, md[1]

Because obesity is a powerful factor for the development of type 2 diabetes, genes involved in the pathogenesis of obesity might also play a role in type 2 diabetes (1). In obese subjects, heterozygous carriers of a pentanucleotide insertion in the 3′-untranslated region (UTR) of the *LEPR* gene had lower serum insulin concentrations than subjects homozygous for the more common deletion allele (2,3). Furthermore, in a population-based prospective study of a small number of healthy subjects, carriers of the insertion allele of the 3′-UTR *LEPR* polymorphism had a reduced risk of incident type 2 diabetes over 4 years compared with subjects with the deletion allele; the former also had lower fasting serum insulin levels than the latter (4).

Plasma adiponectin levels are reduced in patients with obesity (5) and type 2 diabetes (6). Data from two linkage studies suggest that genetic variants of the *LEPR* gene are associated with insulin resistance and type 2 diabetes (7,8). Furthermore, two single nucleotide polymorphisms (SNPs) in the *APM1* gene, a silent *T* to *G* substitution in exon 2 (*45T/G*) and a *G* to *T* substitution in intron 2 (*276G/T*), have been found to be

associated with type 2 diabetes in Caucasian and Japanese subjects (9–11).

Our aim was to evaluate the role of the *I/D-LEPR* polymorphism and the *SNP45-APM1* in the development of impaired glucose tolerance (IGT) and type 2 diabetes in the Mexico City Diabetes Study (12), a prospective population-based study with a high prevalence of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Anthropometric and clinical parameters were measured as described elsewhere (12). At baseline and follow-up, case subjects were classified as having IGT or type 2 diabetes, according to American Diabetes Association criteria (13). The present analysis is based on the 1,485 subjects in whom DNA was available. In 1993, a first follow-up screening was performed; in 1997, 1,141 subjects completed a second follow-up examination. The protocol was approved by the American British Cowdray Hospital in Mexico City, and all the subjects gave informed consent.

DNA was isolated from peripheral blood leukocytes (14), and a fragment in the *LEPR* of 114 (*D/D*) or 119 (*I/I*) bp was generated by PCR using the forward

primer 5′-CATGCCTCAATTCCAAAC-3′ and reverse primer 5′-GAGAGAACAAACAGACAACATT-3′. Amplification was performed using 200 ng DNA and 1 μmol/l of each primer using a GeneAmp PCR-System 2400 (Perkin-Elmer, Norwalk, CT). Then, amplicons were analyzed by single-strand conformation polymorphism, performing an electrophoresis on 6% polyacrylamide gel with 10% glycerol at room temperature and silver stained. Fragments showing variations were reamplified by PCR and electrophoresed onto 3.5% agarose gels. A difference of 5 bp between the carriers of the insertion allele (*D/I* or *I/I*) and carriers of the deletion allele (*D/D*) was revealed.

A DNA fragment of 264 bp was generated by PCR using the primer 5′-TGTGTGTGTGGGGTCTGTCT-3′ and 5′-TTCTCACCCTTCTCACCAGG-3′ for *SNP45-APM1*. PCR was performed using 100 ng DNA and 0.2 μmol/l of each primer. Then, PCR products were digested with *Bsp*H I enzyme and electrophoresed on 6% polyacrylamide gel and silver stained. Wild-type allele (*T/T*) was digested in fragments of 158 and 106 bp, whereas mutated alleles (*G/G*) were not digested (264 bp).

Statistical analysis

Logistic regression was carried out by standard methods; results are expressed as the odds ratio (OR) with 95% CIs. Genetic data are presented according to both genotype and allele frequency. χ^2 analysis was used to test for Hardy-Weinberg equilibrium within the normal glucose tolerance (NGT), IGT, and type 2 diabetic groups.

RESULTS — The distribution of the *I/D-LEPR* genotypes was in Hardy-Weinberg equilibrium in all groups ($\chi^2 = 0.17$, $P = $ NS). The *I/I* genotype tended to decrease across IGT and type 2 diabetes (as did the allele frequency), but the trend fell short of statistical significance (Table

Table 1—3'-UTR *I/D polymorphism of the* **LEPR** *gene and* SNP45 *of the* APM1 *gene according to glucose tolerance status and diabetes progression*

	NGT	IGT	Type 2 diabetes	χ^2, P	Nonprogression	IGT progression	Type 2 diabetes progression	χ^2, P
LEPR genotype frequency								
D/D	606 (69)	157 (72)	288 (73)	7.6, 0.11	413 (68)	67 (71)	84 (78)	6.5, 0.17
I/D	234 (27)	56 (26)	103 (26)		167 (27)	26 (27)	21 (20)	
I/I	33 (4)	4 (2)	5 (1)		29 (5)	2 (2)	2 (2)	
LEPR allele frequency								
D	1,446 (83)	370 (88)	679 (85)	4.1, 0.12	995 (82)	160 (84)	189 (88)	6.1, 0.046
I	300 (17)	64 (15)	113 (15)		225 (18)	30 (16)	25 (12)	
APM1 genotype frequency								
T/T	582 (67)	145 (67)	262 (66)	4.9, 0.29	414 (68)	61 (64)	72 (67)	1.3, 0.86
T/G	261 (30)	59 (27)	123 (31)		176 (29)	32 (34)	31 (29)	
G/G	30 (3)	13 (6)	11 (3)		19 (3)	2 (2)	4 (4)	
APM1 allele frequency								
T	1,425 (82)	349 (80)	647 (82)	0.4, 0.83	1,004 (82)	154 (81)	147 (79)	1.3, 0.51
G	321 (18)	85 (20)	145 (18)		214 (18)	36 (19)	39 (21)	

Data are *n* (%) unless otherwise indicated. Nonprogression refers to subjects with NGT at baseline and both follow-up visits. IGT progression refers to subjects with NGT at baseline and IGT at either or both follow-up visits. Type 2 diabetes progression refers to subjects with NGT or IGT at baseline and type 2 diabetes at either or both follow-up visits.

1). The *SNP45-APM1* genotypes also were in Hardy-Weinberg equilibrium in all three groups ($\chi^2 = 0.4$, P = NS). No difference was found in either genotype distribution or allele frequency across glucose tolerance status or for obesity and body weight.

Over 7 years of follow-up, 95 subjects progressed to IGT and 107 developed type 2 diabetes. The *I* allele was significantly less expressed in subjects who progressed to type 2 diabetes than in subjects who remained normal glucose tolerant ($\chi^2 = 5.8$, P = 0.016). In contrast, no association was found between the *SNP45-APM1* and incident IGT or type 2 diabetes (Table 1). For type 2 diabetes, incidence was twice as high in DD (1.6% per year) than II (0.8% per year).

In a multiple logistic regression model adjusting for sex, age, BMI, waist circumference, blood pressure, triglyceride, HDL cholesterol, and 2-h plasma insulin concentrations, the presence of the *I* allele of *LEPR* was a significant predictor of the combined outcome of type 2 diabetes or IGT (OR 0.66 [95% CI 0.45–0.96]) together with BMI (1.32 [1.16–1.50] for every three BMI units), systolic blood pressure (1.17 [1.05–1.29] for every 10 mmHg), 2-h plasma insulin (1.06 [1.04–1.09] for every 60 pmol/l), or HDL cholesterol 0.87 [0.79–0.97] for every 5 mg/dl). In the same model, the *SNP45-APM1* polymorphism was unrelated to worsening glucose tolerance.

CONCLUSIONS — The main findings of the present study were that the *1)* I allele of the 3′UTR-LEPR gene signaled a reduced risk of development of diabetes, *2)* the *SNP45-APM1* did not predict incident diabetes, and *3)* no effect of either *LEPR* or *APM1* polymorphism on obesity and body weight was found in this population.

Several polymorphisms in *LEPR* have been identified (15–18), and recently, two studies carried out in obese subjects reported that heterozygous carriers of the *I/D-LEPR* had lower serum insulin concentrations than *DD* carriers (2,3); this finding has been interpreted to indicate that defects of *LEPR* could play a role in the etiology of diabetes rather than obesity (2). In agreement with this suggestion, Lakka et al. (4) described an association between *I/D*-LEPR and risk of type 2 diabetes in a prospective, case-control study in healthy men.

In our general population, the association between *I/D-LEPR* and prevalent IGT or type 2 diabetes did not reach statistical significance, and we did not find significant associations with other features of the metabolic syndrome; however, the *I* allele did predict incident diabetes independently of confounders, in agreement with data from Lakka et al. (4).

The mechanism underlying the association between this polymorphism and the risk of diabetes is not clear. Leptin receptors are present on pancreatic β-cells, whereby leptin could modulate glucose-induced insulin secretion (15,16). It has been suggested that *I/D-LEPR* could affect RNA stability and, possibly, the rate of degradation and/or translation of mRNA (3,17). However, direct data on the effects of the polymorphism on leptin action or insulin secretion are not available.

Recently, *SNP45* and -276, in exon 2 and intron 2 of *APM1*, respectively, have been found to be associated with type 2 diabetes in Japanese patients (9), but this association has not been confirmed in French or Swedish populations (18,19). We therefore evaluated the role of *SNP45-APM1* on the susceptibility to diabetes and/or the linkage with *I/D-LEPR*. The current results were negative on the prediction of diabetes. However, it is important to note that in complex traits such as diabetes, the absence of association does not necessarily indicate a lack of effect because the susceptibility to disease may be conferred by combinations of variants rather than a single SNP.

References
1. Albu JB, Curi M, Shur M, Murphy L, Matthews DE, Pi-Sunyer FX: Systemic resistance to the antilipolytic effect of insulin in black and white women with visceral obesity. *Am J Physiol* 277:E551–E560, 1999
2. Francke S, Clement K, Dina C, Inoue H, Behn P, Vatin V, Basdevant A, Guy-Grand B, Permutt MA, Froguel P, Hager J: Ge-

netic studies of the leptin receptor gene in morbidly obese French Caucasian families. *Hum Genet* 100:491–496, 1997

3. Oksanen L, Kaprio J, Mustajoki P, Kontula K: A common pentanucleotide polymorphism of the 3′-untranslated part of the leptin receptor gene generates a putative stem-loop motif in the mRNA and is associated with serum insulin levels in obese individuals. *Int J Obes Relat Metab Disord* 22:634–640, 1998

4. Lakka HM, Oksanen L, Tuomainen TP, Kontula K, Salonen JT: The common pentanucleotide polymorphism of the 3′-untranslated region of the leptin receptor gene is associated with serum insulin levels and the risk of type 2 diabetes in nondiabetic men: a prospective case-control study. *J Intern Med* 248:77–83, 2000

5. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999

6. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000

7. Vionnet N, Hani El-H, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. *Am J Hum Genet* 67:1470–1480, 2000

8. Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L, Blangero J, Comuzzie AG: Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci U S A* 97:14478–14483, 2000

9. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T: Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 51:536–540, 2002

10. Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, Trischitta V, Doria A: A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes* 51:2306–2312, 2002

11. Stumvoll M, Tschritter O, Fritsche A, Staiger H, Renn W, Weisser M, Machicao F, Häring H: Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. *Diabetes* 51:37–41, 2002

12. Nannipieri M, Posadas R, Williams K, Politi E, Gonzales-Villalpando C, Stern MP, Ferrannini E: Association between polymorphisms of the atrial natriuretic peptide gene and proteinuria: a population-based study. *Diabetologia* 46:429–432, 2003

13. The 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* 25 (Suppl. 1):S5–S20, 2002

14. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215, 1988

15. Kieffer TJ, Heller SR, Leech CA, Holz GG, Habener JF: Leptin suppression of insulin secretion by activation of ATP-sensitive K^+ channels in pancreatic β-cells. *Diabetes* 46:1087–1093, 1997

16. Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M: Expression of the leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 46:313–316, 1997

17. Brown CY, Lagnado CA, Goodall GJ: A cytokine mRNA-destabilizing element that is structurally and functionally distinct from A+U-rich elements. *Proc Natl Acad Sci U S A* 93:17721–17725, 1996

18. Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, Boutin P, Vaxillaire M, Lepretre F, Dupont S, Hara K, Clement K, Bihain B, Kadowaki T, Froguel P: Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet* 11:2607–2614, 2002

19. Gu HF, Abulaiti A, Östenson CG, Humphreys K, Wahlestedt C, Brookes AJ, Efendic S: Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (*APM1*) gene are associated with type 2 diabetes in Swedish Caucasians. *Diabetes* 53 (Suppl. 1):S31–S35, 2004