Brief Genetics Report

Positional Candidate Gene Analysis of Lim Domain Homeobox Gene (Isl-1) on Chromosome 5q11-q13 in a French Morbidly Obese Population Suggests Indication for Association With Type 2 Diabetes

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The Lim domain homeobox gene (Isl-1) is a positional candidate gene for obesity that maps on chromosome 5q11-q13, a locus linked to BMI and leptin levels in French Caucasians. Isl-1 might be involved in body weight regulation and glucose homeostasis via the activation of proglucagon gene expression, which encodes for glucagon and glucagon-like peptides. By mutation screening of 72 obese subjects, we identified three single-nucleotide polymorphisms (SNPs) in the Isl1 gene. The allele frequencies in the morbidly obese group did not differ from that of the control group. In the obese group, the −47G allele was associated with a decreased risk of type 2 diabetes (odds ratio 0.41, *P* = 0.019). The AG bearers displayed a higher maximal BMI than the AA bearers in the whole obese group (*P* = 0.026) as well as in the type 2 diabetic obese subgroup (*P* = 0.014). In obese families, this allele was not preferentially transmitted from heterozygous parents to their obese siblings, indicating that Isl-1 does not contribute to the linkage with obesity on 5cen-q. However, in French Caucasian morbidly obese subjects, the Isl1–47A→G SNP may modulate the risk for type 2 diabetes and may increase body weight in diabetic morbidly obese subjects. *Diabetes* 51:1640–1643, 2002

Positional candidate gene analysis has been proposed as a powerful tool to identify genetic determinants of multifactorial diseases (1). A genome-wide scan of French obese Caucasian families has identified three loci linked with obesity-related traits on chromosome 2p, 10p, and 5cen-q (2). The Lim domain homeobox gene Isl-1 at the 5q11-q13 locus might be a candidate gene for obesity and associated diseases. An intragenic microsatellite in the Isl-1 gene was linked with serum leptin levels and BMI in French families with morbid obesity (3). Isl-1 protein might play a role in body weight and glucose homeostasis by transactivating the proglucagon gene encoding glucagon and the glucagon-like peptides (GLPs) GLP-1 and GLP-2 (4). GLP-1 receptor (GLP-1R) and GLP-2R are highly expressed in hypothalamic regions involved in feeding behavior regulation (5,6). Although rat GLP-2 is a more potent anorexigenic than GLP-1 (6), centrally injected GLP-1 also inhibits food intake in fasted or neuropeptide Y–treated rats (5). Conversely, GLP-1R$^{-/-}$ mice are not obese and display normal satiety and impaired glucose tolerance (7,8).

To evaluate the putative role of Isl-1 in obesity and associated insulin resistance, we screened its gene for mutation in 72 obese subjects linked to the 5cen-q locus (2). Association studies performed in the French morbidly obese cohort (9) revealed a trend toward association between the −47A→G single-nucleotide polymorphism (SNP) and the maximal reached BMI. The contribution of this SNP in the linkage previously reported (2) was investigated in the French obese families using a transmission disequilibrium test (TDT).

Three SNPs were identified in the Isl-1 gene: −495A→G and −47A→G in the 5′ region (+1 design the ATG codon) and a silent mutation, P168P (CCA→CCG), in the fourth exon. The P168P SNP was previously identified in both French and Japanese type 2 diabetic patients, and the −47A→G SNP was found in French patients (10,11). We did not find the Q310X nonsense mutation identified in a type 2 diabetic Japanese family (11). Genotype frequencies and allele distributions were not different between the morbidly obese and control groups (Tables 1 and 2). Allele frequencies did not deviate from the Hardy-Weinberg equilibrium, and no linkage disequilibrium was observed between the SNPs (data not shown). No −47GG carriers were found in either group. In the whole morbidly obese group (n = 579), the AG genotype was more frequent in normoglycemic than in type 2 diabetic patients (P = 0.017), and it was moderately associated with a lower risk

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Additional information can be found in an online appendix at http://diabetes.diabetesjournals.org.

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GLP, glucagon-like peptide; GLP-R, GLP receptor; OR, odds ratio; SNP, single-nucleotide polymorphism; TDT, transmission disequilibrium test.

TABLE 1
Genotype frequencies of the Isl-1 SNPs in the morbidly obese and control groups

SNPs	Morbidly obese group: Morbidly obese normoglycemic and type 2 diabetic	Morbidly obese group: Morbidly obese type 2 diabetic subgroup 1	Morbidly obese group: Morbidly obese normoglycemic subgroup 2	Control group: Nonobese normoglycemic	χ^2 Pearson P values (Bonferoni P values)
−47A→G					
n	579	360	219	213	
AA	94.6	96.4	91.8	93.9	0.017 (0.034)
AG	5.4	3.6*	8.2*	6.1	
GG	0	0	0	0	
−495A→G					
n	336	191	146	221	
AA	74.2	74.4	74.0	71.5	>0.05
AG	23.7	23.0	24.7	27.1	
GG	2.1	2.6	1.3	1.4	
P168P					
n	362	203	159	225	
AA	55.5	56.2	54.7	52.0	>0.05
AG	32.3	32.5	32.1	40	
GG	12.2	11.3	13.2	7.6	

Data are %. For the −47A→G SNP, χ^2 tests were performed between all obese patients and the control group ($P = 0.683$), between type 2 diabetic, normoglycemic, and the nonobese normoglycemic control group ($P = 0.056$), and between type 2 diabetic and normoglycemic groups ($P = 0.017$). The latter P value is reported in the table, and Bonferoni corrections for multiple testing were specified in parentheses. For the two other SNPs, the same calculations were performed between the different subject groups and subgroups, and the P values were >0.05. *$P = 0.017$.

of type 2 diabetes (odds ratio [OR] 0.41, $P = 0.019$). Interestingly, using the type 2 diabetes "large" criteria to define affected status (12), we found that the −47G allele is transmitted to eight obese normoglycemic versus four obese type 2 diabetic siblings ($P = 0.388$). The AG patients displayed a higher maximal BMI reached during adult life than the AA carriers ($P = 0.026$) (Tables 3 and 4). Among the type 2 diabetic morbidly obese patients (subgroup 1, $n = 360$), the AG carriers had a higher actual BMI ($P = 0.026$), a higher maximal BMI ($P = 0.014$), and a higher Z score for BMI ($P = 0.033$) than the AA carriers (Tables 3 and 1). Among the morbidly obese normoglycemic subjects (subgroup 2, $n = 219$, $P > 0.05$), no differences were observed between AG and AA carriers. Corrected P values for multiple testing were specified in Tables 1, 2, and 3.

The contribution of the −47A→G SNP to the linkage at the 5cen-q11 locus was investigated by a familial association study in the French obese families (2). Using the BMI and the serum leptin levels as binary traits, the −47G allele transmission from heterozygous parents to their affected children did not deviate from the Mendelian expectancies of 50% when no linkage is present (data not shown).

The human chromosome 5cen-q locus, encompassing the Isl-1gene, was linked to BMI and leptin levels (2,3) and, recently, to type 2 diabetes (13,14), suggesting a probable genetic link between obesity and type 2 diabetes, two tightly related metabolic diseases. Therefore, we hypothesized that genetic variations in Isl-1, a positional candidate for obesity and related diseases, might affect both food intake and glucose homeostasis by modulating the

TABLE 2
Allele distribution of the Isl-1 SNPs in the morbidly obese and control groups

SNPs	Morbidly obese group: Morbidly obese normoglycemic and type 2 diabetic	Morbidly obese group: Morbidly obese type 2 diabetic subgroup 1	Morbidly obese group: Morbidly obese normoglycemic subgroup 2	Control group: Nonobese normoglycemic subjects	χ^2 Pearson P values (Bonferoni P values)
−47A→G					
n	1,158	720	438	426	
A	1,127	707	420	413	0.030 (0.060)
G	31	13*	18*	13	
−195A→G					
n	674	382	292	442	
A	580	328	252	376	>0.05
G	94	54	40	66	
P168P					
n	724	406	318	450	
A	519	294	225	325	>0.05
G	205	112	93	125	

As for the genotype frequencies comparisons, χ^2 tets suggests a significant difference of allele distribution between morbidly obese type 2 diabetic subgroup 1 and morbidly obese normoglycemic subgroup 2 ($P = 0.030$). Data are *n*.

TABLE 3
Obesity-related phenotypes according to the −47A→G genotype in the morbidly obese group and in the type 2 diabetic subgroup

	A/G	A/A	Wicoxon/Kruskal-Wallis (rank sums) and *P* values (Bonferoni *P* values)
Morbidly obese patients (n = 579)			
n	31	548	
BMI (kg/m^2)	50.3 ± 12.8	46.6 ± 7.3	0.349 (0.698)
Maximal BMI (kg/m^2)	55.3 ± 14.8	49.4 ± 8.1	0.026 (0.052)
Z score of BMI	7.4 ± 3.4	6.4 ± 2.4	0.159 (0.318)
Morbidly obese type 2 diabetic patients (n = 360)			
n	13	347	
BMI (kg/m^2)	56.2 ± 16.5	46.7 ± 7.5	0.026 (0.052)
Maximal BMI (kg/m^2)	60.9 ± 19.2	49.6 ± 8.3	0.014 (0.028)
Z score of BMI	8.8 ± 4.6	6.2 ± 2.4	0.033 (0.066)

Data are means ± SD.

production of the GLPs (4). The prevalence of the three SNPs detected was similar in case and control subjects, but careful analyses suggested that the −47G rare allele may protect morbidly obese subjects from type 2 diabetes (OR = 0.41). Additionally, heterozygous parents from French obese families more frequently transmitted the −47G allele to their obese offspring if they were normoglycemic. However, the small number of obese families with a type 2 diabetic history did not give us enough power to reach statistical significance. Moreover, in the morbidly obese type 2 diabetic subgroup, the G allele carriers exhibited an 11.3 kg/m^2 increase in their maximal BMI, a 9.5 kg/m^2 increase of their actual BMI, and a 2.6-unit increase of their *Z* score for BMI. Although we have subdivided the morbidly obese group according to the type 2 diabetes status, the *P* values corrected for multiple comparisons close the significance. Such effects were not found in the morbidly obese normoglycemic or control groups (data not shown).

Our data suggest that the −47A→G SNP might play a protective role against type 2 diabetes in morbidly obese patients, but analysis of larger populations are certainly needed to ascertain the role of this SNP. Obesity is a major risk factor for type 2 diabetes, which has a prevalence that is positively correlated with BMI. In the French morbidly obese cohort (BMI >40 kg/m^2), the type 2 diabetes prevalence remains remarkably constant at ~30%, as most of the middle-aged patients keep a normal glucose tolerance. Therefore, one may postulate that protective factors might delay the occurrence of type 2 diabetes and thus "contribute" to reach the highest degree of obesity. In the type 2 diabetic subgroup, the −47G rare allele carriers reached the highest levels of BMI, suggesting that they have a higher set point for chronic hyperglycemia breakout. Although insulin gene expression does not require the Isl-1 protein (15), Isl-1 transactivates the proglucagon gene promoter (4). In healthy humans, GLP-1 improves insulin-independent glucose disposition and glucose tolerance by stimulating insulin gene transcription and insulin release (16). Several pharmacological studies reported the effects of GLP-1 analogs in the treatment of type 2 diabetes (17). Thus, SNPs modifying the activity or expression of Isl-1, a glucagon gene transactivator, might modulate the risk of type 2 diabetes. Recently, the insulin gene variable number tandem repeat class I allele was reported to increase insulin levels in obese children, whereas the class III allele contributes to the risk of type 2 diabetes by lowering insulin promoter activity (18). A nucleotide-nucleotide BLAST search at the National Center for Biotechnology Information (NCBI) (available on-line at http://www.ncbi.nlm.nih.gov/blast/), revealed that the Isl-1 gene 5′ untranslated region is highly conserved between humans and rodents (>90%). Although the −47A→G SNP is not located in an obvious nuclear binding site, it might play a functional role in mRNA translational or stability properties, which require complex experiments to be functionally characterized.

In conclusion, we screened the Isl-1 gene in obese

TABLE 4
Clinical data for the extended morbidly obese group (n = 579), the type 2 diabetic and normoglycemic sub-groups (n = 579), and the control group (n = 225)

	Extended morbidly obese group			Control group
	Morbidly obese normoglycemic and type 2 diabetic	Morbidly obese type 2 diabetic subgroup 1	Morbidly obese normoglycemic subgroup 2	Nonobese normoglycemic
n	579	360	219	225
Age at diagnosis (years)	47.3 ± 12.7	49.7 ± 11.6	43.3 ± 13.3	59.8 ± 11.9
BMI (kg/m^2)	46.8 ± 7.8	47.0 ± 8.2	46.4 ± 7.0	22.7 ± 2.3
Max BMI (kg/m^2)	49.8 ± 8.7	50.0 ± 9.1	49.3 ± 7.9	—
Z score of BMI*	6.4 ± 2.5	6.3 ± 2.5	6.6 ± 2.4	−0.51 ± 0.54
Sex ratio (F/M)	439/140	250/110	189/30	133/92

Data are means ± SD. **Z* score of the BMI was defined as the variation of the BMI when compared with age- and sex-matched French reference population.

patients from families linked to the 5cen-q locus. We found no obvious evidence that the −47A→G SNP might contribute to the previously reported linkage. However, we report that this SNP might reduce the risk for type 2 diabetes in morbidly obese patients. Further analyses in additional obese population samples will be helpful to clarify this issue.

RESEARCH DESIGN AND METHODS

According to the nonparametric-affected sib pair linkage results, we selected 72 obese patients (BMI >27 kg/m^2), one per affected sib pair, presenting a mean proportion of marker alleles shared identical-by-descent (IBD) $\pi > 0.5$ (2). We then screened the proximal 5′ region, the six coding exons, and the exon-intron junctions of the Isl-1 gene by direct sequencing (19) (primer sequences and amplification conditions are available in the online appendix at http://diabetes.diabetesjournals.org). Association studies were initially carried out in a first set of 362 unrelated morbidly obese subjects (mean BMI 47.3 ± 7.4, women-to-men sex ratio = 284:78) and 225 unrelated nonobese normoglycemic control subjects (mean BMI 22.7 ± 2.3, women-to-men sex ratio = 133:92). Because positive results were initially obtained with the −47A→G SNP (data not shown), we extended the analysis for this SNP to 579 unrelated morbidly obese patients. We divided this group into subgroups 1 and 2 according to the type 2 diabetes "large" status (12). Clinical data for the different groups and subgroups are reported in Table 4. Genotyping of the three identified Isl-1 SNPs (−495A→G, −47A→G, and P168P) was performed by PCR–restriction fragment–length polymorphism with the following restriction enzymes: *Alu*I, *Pvu*II, and *Mbo*I (New England Biolabs, Beverly, MA), respectively. Categorical variables were compared between groups using the χ^2 test. Because the obesity-related phenotypes were not normally distributed and the number of −47G allele carriers was small, the nonparametric Wilcoxon/Kruskal-Wallis test was used for continuous variables.

To assess the role of the −47A→G SNP in linkage to the 5cen-q locus, we performed a TDT in 158 nuclear families, including a proband with a BMI >40 kg/m^2 and at least one affected sibling with a BMI >27 kg/m^2 (2). The TDT evaluates the −47G rare allele transmission from heterozygous parents to affected siblings. We used the TDTLIKE α test version, which computed the TDT-like likelihood ratio statistics based on the Terwilliger algorithm (20). Because many parents were missing, we also used a sib-TDT implemented in the XDT program (21). The "affected" status was declared when the BMI exceeded the threshold value of 27 kg/m^2 (2) and when the subjects presented the diabetic "large" criteria (12). In addition, we also analyzed the serum leptin values as a dichotomous trait, assuming that a subject was affected when his or her serum leptin level exceeded the mean of our sample (mean leptin level for women = 36.8 ± 17.5 and for men = 17.4 ± 10.9).

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