Functional Variants of the HMGA1 Gene and Type 2 Diabetes Mellitus

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TYP E 2 DIABETE S MELLITUS (DM) is a common metabolic disorder that affects nearly 250 million people worldwide, and is associated with major diabetes-related complications, including retinopathy, nephropathy, neuropathy, and cardiovascular disease. Type 2 DM is a complex disease in which both genetic and environmental factors interact to produce hyperglycemia. Insulin resistance in muscle, liver, and adipose tissue is a major feature of most patients with type 2 DM, making these tissues resistant to both endogenous and exogenous insulin.

There is considerable evidence that heredity is a major contributor to the insulin resistance of type 2 DM. Initially, among those destined to develop type 2 DM, the beta cells of the pancreas compensate with increased insulin secretion; however, the pancreatic beta cells eventually become exhausted by the demands of hyperglycemia.

For editorial comment see p 938.

Context High-mobility group A1 (HMGA1) protein is a key regulator of insulin receptor (INSR) gene expression. We previously identified a functional HMGA1 gene variant in 2 insulin-resistant patients with decreased INSR expression and type 2 diabetes mellitus (DM).

Objective To examine the association of HMGA1 gene variants with type 2 DM.

Design, Settings, and Participants Case-control study that analyzed the HMGA1 gene in patients with type 2 DM and controls from 3 populations of white European ancestry. Italian patients with type 2 DM (n=3278) and 2 groups of controls (n=3328) were attending the University of Catanzaro outpatient clinics and other health care sites in Calabria, Italy, during 2003-2009; US patients with type 2 DM (n=970) were recruited in Northern California clinics between 1994 and 2005 and controls (n=958) were senior athletes without DM collected in 2004 and 2009; and French patients with type 2 DM (n=354) and healthy controls (n=50) were enrolled at the University of Reims in 1992. Genomic DNA was either directly sequenced or analyzed for specific HMGA1 mutations. Messenger RNA and protein expression for HMGA1 and INSR were measured in both peripheral lymphomonocytes and cultured Epstein-Barr virus–transformed lymphoblasts from patients with type 2 DM and controls.

Main Outcome Measures The frequency of HMGA1 gene variants among cases and controls. Odds ratios (ORs) for type 2 DM were estimated by logistic regression analysis.

Results The most frequent functional HMGA1 variant, IVS5-13insC, was present in 7% to 8% of patients with type 2 DM in all 3 populations. The prevalence of IVS5-13insC variant was higher among patients with type 2 DM than among controls in the Italian population (7.23% vs 0.43% in one control group; OR, 15.77 [95% confidence interval {CI}, 8.57-29.03]; P<.001 and 7.6% vs 0% among controls [OR, 1.64 [95% CI, 1.05-2.57]; P=.03). In the French population, the prevalence of IVS5-13insC variant was 7.6% among patients with type 2 DM and 0% among controls (P=.046). In the Italian population, 3 other functional variants were observed. When all 4 variants were analyzed, HMGA1 defects were present in 9.8% of Italian patients with type 2 DM and 0.6% of controls. In addition to the IVS5 C-insertion, the c.310G>T (p.E104X) variant was found in 14 patients and no controls (Bonferroni-adjusted P=.01); the c.*82G>A variant (rs2780219) was found in 46 patients and 5 controls (Bonferroni-adjusted P<.001); the c.*369del variant was found in 24 patients and no controls (Bonferroni-adjusted P<.001). In circulating monocytes and Epstein-Barr virus–transformed lymphoblasts from patients with type 2 DM and the IVS5-13insC variant, the messenger RNA levels and protein content of both HMGA1 and the INSR were decreased by 40% to 50%, and these defects were corrected by transfection with HMGA1 complementary DNA.

Conclusions Compared with healthy controls, the presence of functional HMGA1 gene variants in individuals of white European ancestry was associated with type 2 DM.

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lin secretion to overcome resistance to insulin, and normal glucose tolerance is maintained. Hyperglycemia in insulin-resistant individuals develops when beta cells fail to compensate. However, despite extensive investigations, including studies of candidate genes and the recent genome-wide association studies, the common genetic causes of insulin resistance remain elusive.

The interaction of insulin with target cells is mediated by the insulin receptor (INSR), a glycoprotein that is located on the plasma membrane and serves a crucial role both in directing insulin to target tissues and initiating the responses of these tissues to the hormone. Insulin-resistant individuals rarely have been described who have point mutations in the coding sequence of the INSR gene, resulting in reduced or absent INSR expression in target tissues. Decreases in INSR content due to insulin-induced receptor downregulation in cells have been described in patients who are obese, insulin resistant, have type 2 DM, and are markedly hyperinsulinemic. However, this decrease is reversible and is due to enhanced INSR degradation.

The architectural transcription factor, high-mobility group A1 (HMGA1), is a small nonhistone basic protein that binds to AT-rich regions of DNA via AT hooks and functions as a specific co-factor for gene activation. We previously found that HMGA1 is a key regulator of INSR gene expression. We previously identified 2 patients with insulin-resistant type 2 DM, who had defects in HMGA1 expression and concomitant decreased INSR expression in muscle, fat, and circulating monocytes. These individuals had normal INSR genes, but had a novel genetic variant (c.369delG) in the 3’ non-coding region of the HMGA1 messenger RNA that contributed to the reduction of messenger RNA half-life and subsequent decline in HMGA1 expression. Epstein-Barr virus (EBV)–transformed lymphoblasts from these patients demonstrated defects in HMGA1 and INSR expression, indicating that the defects observed in vivo were not due to the altered metabolic state of the patients. In addition, the in vitro restoration of HMGA1 RNA and protein expression in these cells normalized INSR gene expression, and restored both INSR protein expression and 125I-insulin binding. Additionally, in mice, we found that the loss of HMGA1 expression (induced by disrupting the HMGA1 gene) considerably decreased INSR expression in the major target tissues of insulin action. Thus, human and animal studies support the concept that functional HMGA1 gene variants decrease INSR expression.

In the present study, we examined DNA from a large number of patients with type 2 DM to determine the prevalence of functional HMGA1 gene variants in this disease. We also conducted case-control studies in 3 populations with the major variant to determine the association of HMGA1 gene variants with type 2 DM.

METHODS

Study Populations

We studied type 2 DM populations in 3 different geographic areas (Table 1). All participants were of non-Hispanic, white European ancestry. The first and largest population consisted of 3278 Italian patients with type 2 DM attending the University of Catanzaro’s outpatient clinics and other health care sites in Calabria, Italy, between September 2003 and December 2009. Diabetes was diagnosed according to the American Diabetes Association criteria based on fasting glucose levels (≥126 mg/dL; to convert to mmol/L, multiply by 0.0555). Fasting plasma glucose was measured in all individuals with no caloric intake for at least 8 hours and, in the absence of unequivocal hyperglycemia, was confirmed by repeat testing. A total of 2844 patients (86.8%) were receiving treatment with oral antidiabetes medications, insulin, or both. Basal insulin was measured in the remaining 434 patients by radioimmunoassay. Patients with type 1 DM were excluded on clinical grounds and, in nonunequivocal cases, on the basis of fasting C-peptide levels and negative islet-related autoantibodies.

Two nondiabetic control groups were studied in the Italian population during the same period. The largest control group consisted of 2544 interviewed controls (control group 1). Several measures were taken to screen for those individuals who may have had prediabetes. First, we only studied individuals who were healthy, without type 2 DM (fasting plasma glucose <100 mg/dL), and unrelated to one another. Second, we also conducted personal interviews to determine that neither these individuals nor their family members had a history of either type 2 DM or related conditions, including hypertension, hyperlipidemia, and premature cardiovascular disease.

The next control group (control group 2) was based on a self-reported medical questionnaire and included 784 individuals who were healthy, without type 2 DM, and unrelated to one another and were screened for the absence of type 2 DM (fasting plasma glucose <100 mg/dL). We recruited this second control group to test the hypothesis that a personal interview would be helpful in screening out patients with prediabetes. Both Italian control groups were identified from the same University of Catanzaro’s outpatient clinics and other health care sites in Calabria as the cases.

Fasting insulin levels were measured in a substudy of selected patients with type 2 DM, who were either carriers or noncarriers of the HMGA1 gene mutation and were not taking medications affecting glucose tolerance. They were matched for age, sex, body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), and age at diagnosis of type 2 DM.

The second type 2 DM population included 970 US white patients with type 2 DM obtained from the Genomic Resource in Arteriosclerosis at the University of California San Francisco (UCSF). These patients were seen in UCSF clinics or in cardiology clinics in Northern California and were recruited between 1994 and 2005. The diagnosis of type 2 DM and the exclusion of type 1 DM were based on the same criteria used in the Italian popu-
lution. Controls were 958 healthy individuals without diabetes who were recruited at the 2004 Huntsman World Senior Games (in St George, Utah) and at the 2009 Summer National Senior Games (in Palo Alto, California). These controls were screened by questionnaire only. We studied athletes at 2 events to obtain sufficient numbers of control individuals for statistical power.

The third type 2 DM population comprised 354 unrelated, white French patients with type 2 DM who were attending the Diabetes Center in Reims, France. The criteria for type 2 DM at the time of the enrollment (1992) were those defined by the National Diabetes Data Group (8-hour fasting plasma glucose >140 mg/dL).24 During the same period, a cohort of 50 healthy, nonobese, white, French controls without diabetes25 were recruited from the same medical institution during investigations of dyslipidemia, in which control individuals without any metabolic disturbances were required.

These studies were approved by ethics committees (Comitato Etico Regionale Calabria, Azienda Ospedaliera “Mater Domini,” Catanzaro; UCSF Committee of Human Research Internal Review Board; and the ethics committee at the Centre Hospitalier-Universitaire, Reims, France) and informed written consent was obtained for all individuals. A prior study23 indicated that there are racial differences in the inheritance of type 2 DM. Thus, to avoid confounders, we only studied one racial group, white persons of European ancestry, which was the major racial group in all of our clinics.

**Variant Analysis**

In all 3 populations, genomic DNA from blood cells was used to analyze the HMGAI gene. In the entire Italian type 2 DM population and the interviewed Italian controls (control group 1), this DNA was used as a template for the polymerase chain reaction (PCR). We amplified the entire promoter region (1541 base pairs), all exons, including the complete 3’ untranslated region (3’-UTR), and splice sites of the human HMGAI gene (GenBank NT_007592.15). The PCR products were purified and sequenced in both sense and antisense directions either by a 3100 or 3730xl DNA Analyzer (Applied Biosystems, Foster City, California), or by the Macrogen company (Seoul, South Korea). Genomic variations were confirmed in 3 separate PCR amplification products.

Because of the relatively small number of patients, we genotyped only the IVS5-13insC variant in the US and French populations with type 2 DM, the noninterviewed Italian controls (control group 2), the US controls, and the French controls. The IVS5-13insC variant was selected because it was by far the most common variant. The fluorescence-based TaqMan allelic discrimination technique (Applied Biosystems) was used. The genotyping reaction was performed on a GeneAmp PCR system 9700, and fluorescence was detected on an ABI PRISM 7700 sequence detector (Applied Biosystems). To verify that the TaqMan and direct sequencing techniques were in agreement, the DNA samples from 192 patients with type 2 DM and 96 controls were directly sequenced for the exon 6 and adjacent introns of the HMGAI gene and showed complete concordance with the TaqMan allelic discrimination technique.

**Table 1. General Characteristics of the 3 Study Populations With or Without Type 2 Diabetes Mellitus (DM)**

<table>
<thead>
<tr>
<th></th>
<th>United States</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Type 2 DM</td>
<td>Control Group 1</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>1828</td>
<td>1293</td>
</tr>
<tr>
<td>Age, mean (SD) y</td>
<td>66.4 (10.1)</td>
<td>66.3 (9.7)</td>
</tr>
<tr>
<td>Mean (SD) [range]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>66.3 (10.5)</td>
<td>66.2 (9.3)</td>
</tr>
<tr>
<td>Males</td>
<td>66.3 (9.5)</td>
<td>66.4 (9.5)</td>
</tr>
<tr>
<td>At diagnosis</td>
<td>57.4 (10.3)</td>
<td>NA</td>
</tr>
<tr>
<td>Females</td>
<td>57.6 (10.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Males</td>
<td>57.2 (10.9)</td>
<td>NA</td>
</tr>
<tr>
<td>BMI, mean (SD) [a]</td>
<td>27.7 (4.5)</td>
<td>24.8 (4.3)</td>
</tr>
<tr>
<td>Mean (SD) [range]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>27.7 (4.8)</td>
<td>24.5 (4.2)</td>
</tr>
<tr>
<td>Males</td>
<td>27.7 (4.4)</td>
<td>24.8 (4.4)</td>
</tr>
</tbody>
</table>

Abbreviation: NA, data not applicable.

[a] The 2-tailed standard t test was used for comparisons of means and the 2-tailed Fisher exact test was used for comparisons of proportions.

[b] Selected on the basis of fasting plasma glucose levels (<100 mg/dL), to convert to mmol/L, multiply by 0.0555 and personal interview.

[c] Selected on the basis of written questionnaire.

[d] Calculated as weight in kilograms divided by height in meters squared.

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Reverse-Transcriptase PCR and Immunoblot Analyses

Total RNA from blood monocytes was used to synthesize complementary DNA and perform reverse-transcriptase PCR (RT-PCR) amplification and hybridization with [32P]-labeled PCR-generated probes for HMGA1, INSR, and RPS9 complementary DNAs. The same complementary DNA was used to perform quantitative RT-PCR using a real-time thermocycler (Eppendorf Mastercycler ep realplex; Eppendorf, Hamburg, Germany). Immunoprecipitation and Western blots of HMGA1 and INSR were performed on cellular lysates and nuclear extracts from blood monocytes of patients and healthy controls as previously described. Antibodies used for these studies included antibody to HMGA1, and antibodies to INSRβ and β-Actin (Santa Cruz Biotechnology, Santa Cruz, California).

Studies of EBV-Transformed Lymphocytes

In the Italian population, cultured EBV-transformed lymphoblasts were obtained from the individuals with type 2 DM and the IVS5-13insC variant, individuals with wild-type type 2 DM, and controls without diabetes. Complementary DNA expression vectors (sense and antisense) for HMGA1 were transiently transfected into these lymphoblasts, and HMGA1 and INSR protein levels were assayed 48 to 72 hours later. 125I-insulin binding to these transfected lymphoblasts was measured 72 hours after transfection.

Statistical Analysis

In conducting a general characterization of the study populations, 2-tailed standard t tests were used for comparisons of means, and 2-tailed Fisher exact tests were used for comparisons of proportions. A logistic regression analysis was used to assess the association of the IVS5-13insC polymorphism with type 2 DM; age, sex, and BMI were added as covariates to the genotype data. The odds ratios (ORs) with 95% confidence intervals (CIs) were further calculated. Generally, a significance level of .05 was set for a type I error in all analyses. Bonferroni adjustment was applied to correct for multiple testing by multiplying single P values by the number of variants identified (n = 25), and maintaining the threshold significance at .05. Calculations were performed using R Statistical Computing software (http://www.r-project.org/) and Haploview software (http://www.broad.mit.edu/mpg/haploview) with respect to the haplotype analysis for the linkage disequilibrium between loci and for the Hardy-Weinberg equilibrium.

Sample size and power calculations were performed using the G*Power statistical software.
tistical software based on preliminary analysis of data in this study. The proportion of controls (n=300) with the HMGA1 variant was 0.037; the proportion of cases (n=300) with the HMGA1 variant was 0.077. Therefore, a sample size of 920 cases and 920 controls was required to obtain a power of at least 0.95 (type II error < .05), with a 2-sided significance level of P < .05.

Primers and PCR cycling conditions are available upon request. Rules for nomenclature for the precise description of sequence variations as compiled by the Human Genome Variation Society were followed (http://www.hgvs.org/mutnomen/).

RESULTS

HMGA1 IVS5-13insC Variant in Patients With Type 2 DM in the Italian Population

Genomic DNA from patients of Italian origin with type 2 DM was sequenced to uncover new functional variants within the human HMGA1 gene. The promoter region, all exons and splice sites, and the 3′-UTR were analyzed. Twenty-five variants were detected; 4 variants were associated with type 2 DM (FIGURE 1 and eTable 1 at http://www .jama.com). No significant linkage disequilibrium between loci was found. The most common variant associated with type 2 DM was the novel heterozygous single-nucleotide insertion identified at position −13 of exon 6 (IVS5-13insC; more precisely, c.136-13insC) (Figure 1 and FIGURE 2 and eTable 1). This mutation was confirmed by cloning and direct sequencing. No homozygotes for this mutation were observed in the present study population, and the HMGA1 IVS5-13insC variant showed slight (but not significant) deviation from Hardy-Weinberg equilibrium (eTable 1).

To determine if this insertion variant had functional effects, we measured levels of HMGA1 messenger RNA and protein in blood monocytes from patients with carrier type 2 DM, those with noncarrier wild-type type 2 DM, and controls. In cells from patients with this variant, HMGA1 messenger RNA expression (mean, 65.30; 95% CI, 63.68-66.92) was significantly reduced compared with both healthy control (mean, 100 [95% CI, 97.29-102.75]; P < .001) and patients with wild-type type 2 DM (mean, 96.29 [95% CI, 94.05-98.54]; P < .001) (FIGURE 3). Similarly, HMGA1 protein expression was significantly reduced in cells from patients with carrier type 2 DM (mean, 63.86; 95% CI, 61.94-65.78) compared with both healthy controls (mean, 100 [95% CI, 97.89-102.16]; P < .001) and patients with wild-type type 2 DM (mean, 65.30; 95% CI, 63.68-66.92; P < .001) (FIGURE 3).

Expression of INSR had similar reductions. The INSR messenger RNA was significantly reduced in cells from patients with the HMGA1 variant (mean, 56.25; 95% CI, 54.80-57.69) compared with both healthy control (mean, 100 [95% CI, 97.42-102.63]; P < .001) and patients with wild-type type 2 DM (mean, 86.36 [95% CI, 84.47-88.25]; P < .001). Moreover, fasting serum insulin levels among those not taking medications known to affect glucose tolerance were similar between patients with wild-type type 2 DM (n=100) and BMI-matched patients with carrier type 2 DM (n=30) (mean [SD], 12.9 [4.6] and 14.8 [5.4] µU/mL, respectively; P = .07) (eTable 2 at http://www.jama.com). Both type 2 DM groups had significantly higher fasting insulin levels than controls (n=100) (mean [SD], 6.07 [1.67] µU/mL; P < .001).

Analysis of cultured EBV-transformed lymphoblasts from patients with type 2 DM and the IVS5-13insC variant revealed that these cells had lower levels of HMGA1 and INSR protein than cells from either patients with wild-type type 2 DM or controls (FIGURE 4). In lymphoblasts from the patients with the IVS5-13insC variant, restoration of HMGA1 protein expression by complementary DNA transfection (in the sense but not antisense direction) restored INSR protein expression (untransfected cells: mean, 35.2 [95% CI, 50.43-59.96]; transfected cells: mean, 90.2 [95% CI, 85.14-95.26]; P < .001) and insulin-binding capacity (untrans-
fected cells: mean, 9.30 [95% CI, 3.37-15.03]; transfected cells: mean, 17.47 [95% CI, 9.00-25.93]; P = .03) (Figure 4).

**Case-Control Association Studies of the IVS5-13insC Variant in the Italian Population**

The frequency of this variant in patients with type 2 DM (n=3278) compared with 2 control populations is shown in Table 2. In the first control group, the healthy individuals without diabetes who had been interviewed and were unrelated to one another had a fasting glucose level lower than 100 mg/dL (n=2544) (control group 1). Of patients with type 2 DM, 7.23% were heterozygous carriers of the IVS5-13insC variant (minor allele frequency was 3.6%) vs 0.43% of individuals in the control group (minor allele frequency was 0.22%; adjusted OR for age, sex, and BMI, 15.77 [95% CI, 8.57-29.03], P < .001).

In the second case-control analysis of the IVS5-13insC variant with the same patients with type 2 DM but with a different control group consisting of individuals with a fasting glucose level of lower than 100 mg/dL (n=784) and who were not interviewed (control group 2), the frequency of the IVS5-13insC variant was 3.3% and was statistically different from the population with type 2 DM (OR, 2.03 [95% CI, 1.51-3.43]; P < .001; Table 2).

**Other HMGA1 Variants in the Italian Population**

We observed 3 other functional HMGA1 gene variants in the Italian population with type 2 DM (Figure 1 and Figure 5). A single nucleotide change in exon 8 at codon 104 c.310G>T (p.Glu104X) (GAG → TAG, Glu → Stop) was observed in 14 patients with type 2 DM (0.43%), but not among controls (Bonferroni-corrected P = .01). This change causes a premature truncation of the protein (variant B, Figure 1 and Figure 5). Two other variants were localized to the 3'UTR of the HMGA1 gene. The 3'UTR sequence integrity is critical for posttranscriptional regulation of the HMGA1 gene.22,27 A variant with a substitution of a G by an A in 82 bases 3' of the stop codon (c.82G>A rs2780219) was identified in 46 patients with type 2 DM (1.4%) and 5 controls (0.2%) (Bonferroni-corrected P < .001) (variant C, Figure 1 and Figure 5). The previously described heterozygous G deletion22 in 369 bases 3' of the stop codon (variant D, Figure 1 and Figure 5; GenBank NT_007592.15) was detected in 24 additional patients with type 2 DM (0.73%), but not among controls (Bonferroni-corrected P < .001). Thus, in the Italian population, 2.56% of patients with type 2 DM had these other variants. These other variants were associated with reduced (mean, 30%-40%) HMGA1 and INSR messenger RNA expression.
RNA and protein expression levels from all patients with mutations compared with both patients with noncarrier type 2 DM and controls (Figure 6).

Studies in the US and French Type 2 DM Populations

Because the IVS5-13insC was the most common variant in the Italian population, we studied its association with type 2 DM in 2 other distinct populations. The fluorescence-based TaqMan allelic discrimination technique revealed that among US patients with type 2 DM, 7.7% were heterozygous for the IVS5-13insC variant compared with 4.7% of individuals in the control group (OR adjusted for age, sex, and BMI, 1.64 [95% CI, 1.05-2.57]; P=.03; Table 2). Thus, in these US controls, the minor allele frequency of the IVS5-13insC variant for patients with type 2 DM was higher than for both the Italian selected (control group 1) and nonselected (control group 2) controls. However, the difference in minor allele frequency between the US patients with type 2 DM and their controls was statistically significant (P=.007).

Analysis using the fluorescence-based TaqMan technique in 354 unrelated French individuals with type 2 DM revealed that 7.6% of these patients were carriers of the IVS5-13insC variant (similar to the Italian and US populations with type 2 DM; P=.046), whereas none of the 50 French controls were carriers of this variant (Table 2).

COMMENT

In the present study, we investigated the status of the nonhistone, architectural transcription factor HMGA1, a major regulator of INSR expression, in 3 populations of patients with type 2 DM. In addition to the original rare variant, c.*369del (reported previously), we found 3 new functional variants of the HMGA1 gene that were associated with decreased INSR gene and INSR protein expression and type 2 DM in a significant proportion of affected individuals of white, European descent. In particular, we identified a novel and relatively common variant having a heterozygous single-nucleotide insertion at position −13 of exon 6 (IVS5-13insC). This variant was associated with reduced levels of HMGA1 messenger RNA and HMGA1 protein, possibly by skipping exon 6 and affecting RNA splicing.

This IVS5-13insC variant was present in approximately 7% of patients with type 2 DM in 3 separate populations of white, European descent (Italy, United States, and France). The absence of homozygotes for this mutation in the present study is consistent with previous observations by us and others indicating that mice homozygous for the Hmgal null allele have reduced fertil-
ity and die prenatally. In the Italian population, in which the entire HMGA1 gene was sequenced, we observed 3 other variants that were associated with decreased HMGA1 gene and HMGA1 protein expression. Thus, when all 4 variants of HMGA1 were combined, approximately 10% of Italian patients with type 2 DM had functional abnormalities of this gene. Because of the low frequency of these variants, we did not test for the 3 less common variants in the US and French populations.

Because insulin downregulates its own receptor, we performed 2 different studies to rule out the possibility that the hyperinsulinemia observed in our patients with HMGA1 gene variants was downregulating the INSR. First, insulin levels in patients with type 2 DM and the most common IVS5-13insC HMGA1 variant were not statistically different from patients with wild-type type 2 DM. Second, cultured EBV-transformed lymphoblasts from patients with the IVS5-13insC HMGA1 variant maintained the INSR defect. Moreover, transfection of the cells from these carrier patients with HMGA1 complementary DNA restored levels of both HMGA1 and the INSR, and restored insulin binding to the cell’s INSR.

One significant difficulty in performing case-control studies of insulin resistance and type 2 DM is defining the appropriate control group. The major problem is that when healthy individuals with normal glucose tolerance test results are investigated, 25% are as insulin resistant (as measured by euglycemic insulin clamp) as patients with type 2 DM. Moreover, many of these individuals, in contrast to insulin-sensitive persons, will subsequently develop type 2 DM and related diseases including hypertension, dyslipidemia, and cardiovascular disease. Thus, without extensive testing it is difficult to exclude individuals with insulin resistance within a control group, and therefore control groups may contain significant numbers of individuals with insulin resistance that will lead to type 2 DM. To reduce the number of persons with insulin resistance in one of our Italian control groups, we conducted personal interviews to obtain more than 2500 healthy individuals who had neither a personal nor family history of type 2 DM and related conditions including hypertension, hyperlipidemia, and premature cardiovascular disease. In our case-control study using these highly screened controls (control group 1), we found that less than 1% had variants of the HMGA1 gene whereas nearly 10% of the type 2 DM population had variants. Thus, there was

**Table 2. IVS5-13insC Variant in the Italian, US, and French Populations**

<table>
<thead>
<tr>
<th></th>
<th>No. (%) With Wild-Type Type 2 DM</th>
<th>No. (%) With IVS5-13insC Variant</th>
<th>Minor Allele Frequency, %</th>
<th>AOR (95% CI)</th>
<th>Adjusted P Value</th>
</tr>
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<tbody>
<tr>
<td><strong>Italian population</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>With type 2 DM</td>
<td>3041 (92.8)</td>
<td>237 (7.2)</td>
<td></td>
<td>3.60</td>
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<tr>
<td>Control group 1</td>
<td>2533 (99.6)</td>
<td>11 (0.4)</td>
<td>0.22</td>
<td>15.77 (8.57-29.03)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Control group 2</td>
<td>758 (96.7)</td>
<td>26 (3.3)</td>
<td>1.66</td>
<td>2.03 (1.51-3.43)</td>
<td>&lt;.001</td>
</tr>
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<td><strong>US population</strong></td>
<td></td>
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<tr>
<td>With type 2 DM</td>
<td>895 (92.3)</td>
<td>75 (7.7)</td>
<td>3.90</td>
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<tr>
<td>Control</td>
<td>913 (95.3)</td>
<td>45 (4.7)</td>
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<td>2.10</td>
<td>1.64 (1.05-2.57)</td>
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<td><strong>French population</strong></td>
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<tr>
<td>With type 2 DM</td>
<td>327 (92.4)</td>
<td>27 (7.6)</td>
<td>3.80</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>50 (100)</td>
<td>0</td>
<td></td>
<td>NA</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; DM, diabetes mellitus; NA, not able to calculate data.

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a high, adjusted OR when these controls were compared with patients with type 2 DM.

To better understand these results with interviewed controls, we also studied a second Italian control group (control group 2) that included 784 unrelated healthy individuals screened only for the absence of type 2 DM and not having a personal interview. Only the common IVS5-13insC was studied. As expected, this control group had a higher percentage of persons with this variant, but the prevalence was still statistically significantly higher among patients with type 2 DM. Similar results were obtained in the US control group, which also did not have personal interviews. The French control group had no carriers of the IVS5-13insC variant, but the population was too small to perform case-control studies. Therefore, for studies of insulin resistance and type 2 DM, our studies highlight the importance of using the selection criteria for obtaining controls without diabetes.

We believe our observation that nearly 10% of individuals with type 2 DM have deleterious variations in the gene encoding HMGA1 has important clinical implications. First, the presence of these variants could serve as an early predictive marker of both insulin resistance and type 2 DM, especially in those individuals who have a family history of type 2 DM and related conditions. Second, the presence of these variants may predict the response to therapy.32 Currently the treatment of type 2 DM has been largely empirical, and it is often difficult to predict responses to a therapeutic agent in any individual patient.7 Because these HMGA1 variants define a specific defect that causes decreased insulin receptors and insulin resistance, patients with type 2 DM and these variants may respond differently to a specific therapy, such as an insulin sensitizer. Third, individuals who have functional HMGA1 variants and type 2 DM may have a different clinical course than other patients with type 2 DM, including differences in the development of macrovascular and microvascular complications. Fourth, the search for new therapies for type 2 DM could include agents that upregulate the expression of HMGA1.

In addition to functional HMGA1 gene variants that are relatively common, we have previously observed 2 rare mechanisms for loss of HMGA1 expression. A hemizygous deletion of the HMGA1 gene (causing reduced HMGA1 expression) was observed in 2 related patients with type 2 DM (mother and daughter) and decreased INSR expression.22 Also, linkage between HMGA1 and INSR expression and type 2 DM was recently reported in 2 unrelated patients who overexpressed a novel HMGA1 pseudogene that caused a marked destabilization of HMGA1 messenger RNA with subsequent loss of INSR expression and clinical insulin resistance.33 Other than HMGA1, abnormalities in only a few genes have been identified that cause insulin resistance. The ectoprotein ENPP1/PC-1, when overexpressed, is an inhibitor of INSR and is associated with insulin resistance and type 2 DM.34 Also variants in this gene, especially one resulting in a K to Q substitution at codon 121, are associated with both insulin resistance and type 2 DM.34 Rare abnormalities of other genes have been reported that cause insulin resistance and type 2 DM, including INSR,13 PPARγ,13 Akt,36 and leptin and its receptor.37

Genome-wide association studies have been performed to identify genes implicated in insulin resistance and type 2 DM susceptibility. To date, the variants at those loci that have been identified account for only a small percentage of diabetes heritability, and it is not clear how these genetic variants affect the susceptibility to type 2 DM.38-41 It is estimated that variants identified in these genome-wide association studies account for less than 10% of the overall estimated genetic contribution to type 2 DM predisposition, with an apparently low OR.42 For example, common genetic variants in TCFT7L2,43 a gene encoding for a high-mobility-group box-containing transcription factor for which the role is not yet understood, represents the most prevalent genetic risk factor for type 2 DM derived from these studies, and has an OR ranging from 1.31 to 1.71.11

In conclusion, our results indicate that variants in the HMGA1 gene are associated with type 2 DM in individuals of white, European descent. Further studies of the HMGA1 gene and its variants, including studies in other racial types, are needed to understand the role of HMGA1 in insulin resistance and type 2 DM.

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