The ORP150 gene that encodes the human oxygen-regulated protein (150 kDa) maps to chromosome 11q23, a region previously reported to be linked to type 2 diabetes and obesity in Pima Indians. This gene was also found to be differentially expressed in global gene expression studies comparing muscle mRNA from insulin-resistant versus insulin-sensitive subjects. Therefore, ORP150 was analyzed as a candidate gene for susceptibility to diabetes. Twelve variants were identified, and three unique representative polymorphisms were genotyped in 1,338 Pima Indians. None of these polymorphisms were associated with diabetes, but two polymorphisms were significantly associated with measures of insulin resistance. These data indicate that ORP150 has a role in insulin action but does not have a major role in determining susceptibility to type 2 diabetes in Pima Indians. Diabetes 51:1618–1621, 2002

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he Pima Indians have the highest reported prevalence of type 2 diabetes of any population in the world (1). Their diabetes is prototypic of this disease and is characterized by obesity, insulin resistance, insulin secretory dysfunction, and increased rates of endogenous glucose production (2). In prospective studies of Pima Indians with normal glucose tolerance, insulin resistance and insulin secretory dysfunction are major predictors of the disease (3). To identify genetic determinants for type 2 diabetes, we have previously completed a genomic scan in 1,338 Pima Indians who had participated in a longitudinal study of diabetes (4). Variance-components methods were used to test for linkage with an age-adjusted diabetes score and with BMI as an estimate of degree of obesity. In multipoint analyses, the strongest evidence for linkage with age-adjusted diabetes (logarithm of odds [LOD] = 1.7) as well as BMI (LOD = 3.6) was on chromosome 11q23–24, centered at 139 cM, with a 1-LOD CI spanning 123–157 cM. Bivariate linkage analysis for the combined phenotype “diabetes” gave the strongest evidence for linkage (LOD = 5.2).

We have also searched for susceptibility genes for type 2 diabetes by oligonucleotide array analysis (Affymetrix). We have compared gene expression in pools of mRNA extracted from skeletal muscle biopsies of nondiabetic insulin-resistant versus insulin-sensitive Pima Indians. Approximately 200 genes and expressed sequence tags (ESTs), of which four mapped to the region of linkage on chromosome 11q23–24, were considered to be potentially differentially expressed between insulin-sensitive and insulin-resistant groups. One gene that mapped to 11q23 was the ORP150 gene. Evidence that ORP150 was involved with susceptibility to diabetes also came from a recent publication showing high levels of ORP150 gene expression in mouse islets and correlation of ORP150 protein levels with insulin secretion (5). Because the human ORP150 gene is positioned at 130 cM, within 6 megabases of the peak of linkage to type 2 diabetes and BMI, we investigated ORP150 as a candidate gene involved in susceptibility to type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects and clinical characteristics. The 1,338 subjects are the same subjects analyzed in our previous genomic scan (4). Diabetes status was determined by a 75-g oral glucose tolerance test, and the results were interpreted according to World Health Organization criteria (6). For detailed metabolic testing, nondiabetic, healthy individuals were admitted to our clinical research ward. Oral glucose tolerance was measured after 2–3 days on a weight-maintaining diet. Subjects ingested 75 g glucose, and blood for plasma glucose and insulin measurements was drawn before ingesting the glucose and at 30, 60, 120, and 180 min thereafter. On a different day, subjects also received an intravenous injection of 25 g glucose over 3 min to measure the acute insulin response, as described elsewhere (7). Body composition was estimated by dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI).

The hyperinsulinemic-euglycemic clamp technique was used to determine basal glucose appearance and insulin-stimulated glucose disappearance (uptake) rates (8). Briefly, insulin was infused to achieve physiologic and maximally stimulating plasma insulin concentrations (137 ± 3 and 2,394 ± 68 μU/ml, respectively) for 100 min for each step. Plasma glucose concentrations were held constant at ~100 mg/dl by a variable 20% glucose infusion. Tritiated glucose was infused for 2 h before the insulin infusion to calculate rates of postabsorptive glucose appearance and glucose disappearance during the lower dose of insulin infusion. All studies were approved by the Tribal Council and the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases.

Differential gene expression. Genechip oligonucleotide arrays (Affymetrix, Santa Clara, CA) containing 6,800 genes and 35,000 ESTs were used to compare global gene expression in pools of mRNA extracted from skeletal muscle biopsies of 17 nondiabetic insulin-resistant Pima Indians (five pools) and 18 insulin-sensitive Pima Indians (five pools). Subjects from each pool were matched for age, sex, and percentage body fat. Percutaneous muscle biopsies were obtained from the quadriceps femoris muscle after local...
anesthesia of skin and fascia with 2% lidocaine. Biopsies were immediately frozen in liquid nitrogen, and RNA was extracted using a ToTally RNA kit (Ambion, Austin, TX).

**Sequencing of ORP150 gene and genotyping of single nucleotide polymorphisms.** All 26 exons, including intron/exon splicing sites, the 5’ and 3’ untranslated regions, and the promoter region (900 bp upstream of the first exon) were sequenced in DNA samples from 39 Pima Indians who were not first-degree relatives of the study subjects. Sequencing was performed using the Big Dye Terminator (Applied Biosystems, Foster City, CA) on an automated DNA capillary sequencer (model 3700; Applied Biosystems). Sequence information for oligonucleotide primers is available on the *Diabetes* website (http://diabetes.diabetesjournals.org/).

Genotyping of single nucleotide polymorphisms (SNPs) in 1,338 subjects was done using the TaqMan assay (Applied Biosystems) for SNPs ORP150-9 and ORP150-12 and pyrosequencing (Pyrosequencing, Uppsala, Sweden) for ORP150-2. The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 9700 (95°C for 10 min) and analyzed on a GeneAmp PCR system 9700 (95°C for 10 min; 95°C for 1 min; and 62°C for 1 min, for 38 cycles), and fluorescence was detected on an ABI Prism 7700 sequence detector (Applied Biosystems). The pyrosequencing reaction was amplified on a GeneAmp PCR system 9700 (95°C for 10 min; then 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min, for 38 cycles, 72°C for 10 min) and analyzed on PSQ96 sequencer (Pyrosequencing).

**Statistical analysis.** Statistical analyses were performed using the statistical analysis system of the SAS Institute (Cary, NC). For continuous variables, the general estimating equation (GEE) procedure was used to adjust for the covariates, including family membership, since some subjects were siblings. Plasma insulin concentrations and rates of glucose disappearance during the low-dose insulin infusion were log-transformed before analyses to approximate a normal distribution. The association of SNP genotypes with diabetes was assessed by analysis of contingency tables.

**RESULTS**

Genechip oligonucleotide arrays were probed with pooled mRNA extracted from skeletal muscle biopsies of 17 nondiabetic insulin-resistant Pima Indians and 18 insulin-sensitive Pima Indians. The ORP150 gene was more highly expressed in the insulin-resistant pools versus insulin-sensitive pools (signal intensity 433 ± 102 and 233 ± 112, respectively; two-tailed t test, *P* = 0.02) (Fig. 1).

To determine whether genetic variation in the ORP150 gene itself caused the higher level of its expression in muscle from insulin-resistant Pima Indians, the ORP150 gene was sequenced in 39 Pima Indian DNA samples to detect variation. Twelve SNPs were identified; their positions within the ORP150 gene are shown in Fig. 2. Based on the genotypes from the 39 Pima Indians, ORP150-1, ORP150-4, ORP150-6, ORP150-8, and ORP150-9 are in 100% linkage disequilibrium with each other, whereas ORP150-5, ORP150-7, ORP150-10, ORP150-11, and ORP150-12 are in nearly 100% linkage disequilibrium with each other. In addition, two of the promoter SNPs, ORP150-2 and ORP150-3, are in 100% linkage disequilibrium. Therefore, ORP150-9, ORP150-12, and ORP150-2 were selected as representative SNPs for each unique group of variants and were genotyped in 1,338 Pima Indians. The allele frequencies for these SNPs are given in Table 1. The genotype distributions for all variants were consistent with Hardy-Weinberg equilibrium. The concordance rate between ORP150-2 and ORP150-9 was 81%; between ORP150-2 and ORP150-12, 74%; and between ORP150-9 and ORP150-12, 81%.

There was no association between the genotypes of ORP150-9, ORP150-12, or ORP150-2 with either type 2 diabetes or BMI in the 1,338 Pima Indians. However, among 227 Pima Indians from this group who had normal glucose tolerance and had undergone a hyperinsulinemic-euglycemic clamp study to assess insulin resistance, both ORP150-2 and ORP150-9 were significantly associated with insulin action at physiologic plasma insulin concentrations (Table 2). In addition, both SNPs were significantly associated with insulin action at maximally stimulating insulin concentrations.

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**FIG. 2.** Variants detected within the *ORP150* gene. Positions of ORP7, ORP9, ORP11, and ORP12 are based on the cDNA sequence NM 006389 (GenBank). Positions of ORP6, ORP8, and ORP10 are based on the genomic contig AP000909 (GenBank) (nucleotides 54815, 31714, and 30393, respectively). Positions of ORP4 and ORP5 in the promoter region are numbered from exon 1B and are based on a published sequence (14), whereas ORP1, ORP2, and ORP3 are based on the genomic contig AP000854 (GenBank) (nucleotides 195825, 195685, and 195681).
TABLE 1
Characteristics of representative ORP150 variants

<table>
<thead>
<tr>
<th>Alleles</th>
<th>ORP150-2</th>
<th>ORP150-9</th>
<th>ORP150-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele frequency</td>
<td>A = 0.20</td>
<td>G = 0.36</td>
<td>C = 0.17</td>
</tr>
<tr>
<td>Position</td>
<td>Promoter (−429)</td>
<td>Exon 19; Ala 745</td>
<td>3’UTR (3700)</td>
</tr>
<tr>
<td>Nucleotide position (GenBank)</td>
<td>195685 (AP000854)</td>
<td>2386 (NM_006380)</td>
<td>3700 (NM_006380)</td>
</tr>
</tbody>
</table>

DISCUSSION

Cellular responses to environmental stresses, such as high temperature and glucose or oxygen deprivation, include induction of de novo protein synthesis of stress-associated proteins such as heat shock proteins, glucose-regulated proteins (GRPs), and oxygen-regulated proteins (ORPs). The rat ORP150 has been purified and characterized (9), and the human cDNA encoding ORP150 has been cloned from a hypoxia-treated astrocytoma library (10). Based on its remarkable sequence homology and shared tissue specificity with known GRPs, ORP150 was proposed to be an endoplasmic reticulum (ER)-associated chaperone involved in protein folding. ORP150 has subsequently been shown to be involved in protection against hypoxia/ischemia-induced neuronal death, regulation of tumor angiogenesis, and acceleration of wound healing by modulated intracellular vascular endothelial growth factor (VEGF) transport (11–13).

ORP150, unlike glucose-regulated proteins GRP78 and GRP94, is markedly expressed in MIN6 β-cells under normal oxygen conditions, suggesting that ORP150 plays a role as an ER chaperone in β-cells (5). It has been proposed that ORP150 may mediate the insulin-synthesizing process by assisting the folding of insulin, prohormone convertase, or other proteins entering secretory granules. Furthermore, decreased serum glucose concentrations severely suppress ORP150 expression in the pancreas, which is consistent with a decrease of serum insulin levels (5). This suggests a tight correlation between ORP150 expression and insulin secretion and indicates that abnormalities of ORP150 could potentially contribute to the genetic predisposition for diabetes and its underlying pathophysiological mechanisms.

The human ORP150 gene maps to a region on chromosome 11q23–24 previously found to be linked to type 2 diabetes and BMI (4). In the present study, we report that ORP150 is more highly expressed in skeletal muscle mRNA from insulin-resistant subjects compared with insulin-sensitive subjects. Although insulin resistance is a strong predictor of type 2 diabetes, our association analysis of three SNPs in ORP150 suggest that this gene, by itself, does not appear to be a major susceptibility gene for type 2 diabetes. Nor was the gene strongly associated with insulin secretion, as measured by the acute insulin response to intravenous glucose (P = 0.07 for ORP150–2), despite findings that MIN6 cells, which constitutively secrete insulin, express very high levels of ORP150 and that ORP150 expression and insulin content are markedly reduced in islets of fasting mice (5).

However, among 227 normal glucose-tolerant Pima subjects who were genotyped, ORP150-2 and ORP150-9 were significantly associated with insulin action at physiologic as well as maximally stimulating insulin levels. ORP150-9 is positioned within exon 19 but does not predict an amino acid substitution; therefore, we do not believe this SNP is a functional variant within this gene, but rather assume that it is in high linkage disequilibrium with a nearby functional variant.

TABLE 2
Association of ORP150-2, ORP150-9, and ORP150-12 with diabetes-related traits in Pima Indians with normal glucose tolerance

<table>
<thead>
<tr>
<th>Allele</th>
<th>n</th>
<th>G/G (175)</th>
<th>G/A + A/A (50)</th>
<th>P</th>
<th>A/A (127)</th>
<th>A/G + G/G (100)</th>
<th>P</th>
<th>G/G (169)</th>
<th>G/C + C/C (58)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>26 ± 1</td>
<td>26 ± 1</td>
<td>—</td>
<td>25 ± 1</td>
<td>27 ± 1</td>
<td>0.19</td>
<td>30 ± 1</td>
<td>32 ± 1</td>
<td>0.22</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td>30 ± 1</td>
<td>31 ± 1</td>
<td>0.88</td>
<td>30 ± 1</td>
<td>32 ± 1</td>
<td>0.19</td>
<td>30 ± 1</td>
<td>32 ± 1</td>
<td>0.22</td>
</tr>
<tr>
<td>2-h plasma glucose (mg/dl)</td>
<td></td>
<td>109 ± 1</td>
<td>110 ± 3</td>
<td>0.85</td>
<td>108 ± 2</td>
<td>111 ± 3</td>
<td>0.62</td>
<td>108 ± 1</td>
<td>111 ± 3</td>
<td>0.57</td>
</tr>
<tr>
<td>Log10 2-h plasma insulin (μU/ml)</td>
<td></td>
<td>2.06 ± 0.02</td>
<td>2.07 ± 0.04</td>
<td>0.69</td>
<td>2.05 ± 0.03</td>
<td>2.06 ± 0.03</td>
<td>0.20</td>
<td>2.06 ± 0.02</td>
<td>2.04 ± 0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>Log10 acute insulin response (μU/ml)</td>
<td></td>
<td>2.34 ± 0.02</td>
<td>2.25 ± 0.04</td>
<td>0.07</td>
<td>2.33 ± 0.02</td>
<td>2.30 ± 0.03</td>
<td>0.44</td>
<td>2.32 ± 0.02</td>
<td>2.33 ± 0.04</td>
<td>0.97</td>
</tr>
<tr>
<td>Log10 glucose disposal for low dose insulin clamp (mg · kg−1 · EMBS · min−1)</td>
<td></td>
<td>0.42 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.007*</td>
<td>0.43 ± 0.01</td>
<td>0.44 ± 0.01</td>
<td>0.08</td>
<td>0.46 ± 0.01</td>
<td>0.44 ± 0.02</td>
<td>0.59</td>
</tr>
<tr>
<td>Glucose disposal for high dose insulin clamp (mg · kg−1 · EMBS · min−1)</td>
<td></td>
<td>9.2 ± 0.1</td>
<td>9.9 ± 0.3</td>
<td>0.009*</td>
<td>9.1 ± 0.2</td>
<td>9.7 ± 0.2</td>
<td>0.002*</td>
<td>9.4 ± 0.2</td>
<td>9.4 ± 0.2</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Data are means ± SE. Because of the low frequency of the rare alleles, for the statistical analyses the homozygotes for each rare allele were combined with the heterozygotes. P values were calculated after adjusting for age and sex for the variable percentage body fat and age, sex, and percentage body fat for the variables 2-h plasma glucose, Log10 2-h plasma insulin, and Log10 glucose disposal for the low-dose and high-dose insulin clamps. The P value for Log10 acute insulin response was calculated after adjusting for age, sex, percentage body fat, and Log10 glucose disposal for the low-dose and high-dose insulin clamps. *Statistically significant. EMBS, estimated metabolic body size.
ORP150-2 is positioned within the putative promoter region. Our microarray studies indicated that ORP150 is more highly expressed in muscle from insulin-resistant subjects; therefore, it is possible that a variation in the ORP150 promoter is responsible for the altered expression. Three distinct mRNAs are transcribed from the ORP150 gene by use of alternative promoters (14). The three messages start with exon 1A, exon 1B, and exon 2. For each transcript, regulatory sequences just upstream of the start site were essential for transcription. ORP150-2 is located at nucleotide position −429 from exon 1B. Although most transcriptional activity has been proposed to be downstream of nucleotide −332, it has been shown that the region upstream of −332 might carry additional transcriptional regulatory sequences (14). However, the MatInspector V2.2 based on TRANSFAC database (http://transfac.gbf.de/TRANSFAC/) did not reveal any known transcriptional factor binding site in the sequence surrounding ORP150-2. In contrast, the region surrounding ORP150-1 matches a transcriptional binding site for Ik2, a potent transcriptional stimulator (15), and ORP150-4 is positioned within one of several putative Sp1 binding sites. Because ORP150-1 and ORP150-4 are in complete linkage disequilibrium with ORP150-9 (and are therefore associated with insulin resistance), it would be worthwhile to conduct further analyses to explore a possible role of these promoter variants on ORP150 expression.

ORP150-5, which is in 100% linkage disequilibrium with ORP150-12, is positioned within a hypoxia-responsive element. This cis-acting element is responsible for selective induction of transcription from exon 1B upon hypoxia or tunicamycin treatment (14). Despite its seemingly functionally important location, ORP150-5 was not associated with diabetes or insulin action in our study.

Based on these data, we suggest that the human ORP150 gene, through its potential role in influencing insulin action, may have a minor contribution in determining, or modifying, type 2 diabetes in Pima Indians. A mechanism by which altered levels of ORP150 could result in insulin resistance, and therefore increase risk for type 2 diabetes, is unknown. The high sequence homology between ORP150 and GRPs may suggest that ORP150 has some role in responding to glucose levels by altering muscle glucose uptake. However, further genetic studies in other populations, as well as functional studies, are needed to better define the relative importance of these genetic variants. It is our hope that once major susceptibility genes for type 2 diabetes are identified, the roles and mechanisms of minor, modifier genes, such as ORP150, will become clearer for this polygenic disease.

REFERENCES