Acknowledgments— This research was supported in part by Grant RR-30 from the General Clinical Research Center Program of the Division of Research Resources, National Institutes of Health (NIH), NIH Research Scientist Development Award K01-MH00303 to R.S.S., and by funds from the Research Network on the Consequences and Determinants of Health Promoting and Health Damaging Behaviors of the John D. and Catherine T. MacArthur Foundation.

Data was managed and analyzed with the Clinfo Data Analysis System of the Clinical Research Unit of Duke University Medical Center (General Clinical Research Centers RR-30).

References

1. Feldberg W, Pike DA, Stubbs WA: On the origin of non-insulin-dependent diabetes. *Lancet* 1:1263–64, 1985
2. Surwit RS, Feinglos MN: Stress and autonomic nervous system in type II diabetes: a hypothesis. *Diabetes Care* 11:83–85, 1988
3. Giugliano D: Morphine, opioid peptides, and pancreatic islet function. *Diabetes Care* 7:92–98, 1984
4. Feldman M, Kiser RS, Unger RH, Li CH: Beta-endorphin and the endocrine pancreas. *N Engl J Med* 308:349–53, 1983
5. Surwit RS, McCubbin JA, Kuhn CM, Cochrane C, Feinglos MN: Differential glycemic effects of morphine in diabetic and normal mice. *Metabolism* 38:282–85, 1989
6. Vermes I, Steinmetz E, Schoorl J, van der Veen EA, Tilders FJH: Increased plasma levels of immunoreactive β-endorphin and corticotropin in non-insulin-dependent diabetes (Letter). *Lancet* 2:725–26, 1985
7. Saad MF, Pettitt DJ, Mott DM, Knowler WC, Nelson RG, Bennett PH: Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. *Lancet* 1:1356–58, 1989
8. Banerji MA, Lebovitz HE: Insulin-sensitive and insulin-resistant variants in NIDDM. *Diabetes* 38:784–92, 1989

Mutations in Insulin-Receptor Gene

Val^{996} Allele in White NIDDM Patients

ANTONIO PORCELLINI, MD
SERGIO COCOZZA, MD
ANTONELLA MONTICELLI, MD
LUIGI PIANESE, BS
GABRIELE RICCARDI, MD
ASSIAMIRA FERRARA, MD
STELIO VARRONE, MD

The insulin-receptor (IR) gene plays a critical role in allowing cells to respond to insulin (1), and insulin resistance is a prominent feature of non-insulin-dependent diabetes mellitus (NIDDM; 2). The involvement of the IR defects in the etiology of diabetes mellitus has been suggested by the findings of mutations in the IR gene in rare patients with genetic syndromes of severe insulin resistance (3–12). Odawara et al. (7) have described a case of young Japanese male with insulin resistance and acanthosis nigricans in which valine is substituted for glycine at position 996 (GTC instead of GGC) in the tyrosine kinase domain of the IR gene. Whether this mutation also contributes to the etiology of the common forms of NIDDM is unknown. Therefore, we determined the prevalence of the Val^{996} allele of the IR gene in a population of white NIDDM patients.

We studied a population of 103 NIDDM patients referred to the Diabetic Clinic of our Medical School. Diagnosis of NIDDM was made according to National Diabetes Data Group criteria. All subjects gave their informed consent to participate in the study. Subjects had a mean ± SE age of 56.9 ± 1.06 yr and a body mass index of 28.12 ± 0.45 kg/m^2. Their diabetes duration was 12.97 ± 0.85 yr. Forty-three were men and 60 were women. We used a modification of the polymerase chain reaction (PCR) procedure that permits the rapid identi-

FROM THE DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY AND PATHOLOGY "L. CALIFANO" AND CENTRO DI ENDOCRINOLOGIA ED ONCOLOGIA SPERIMENTALE, NATIONAL RESEARCH COUNCIL, NAPLES; AND THE INSTITUTE OF INTERNAL MEDICINE AND METABOLIC DISEASE, 2ND MEDICAL SCHOOL, UNIVERSITY OF NAPLES, ITALY.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO SERGIO COCOZZA, MD, DIPARTIMENTO DI BIOLOGIA E PATOLOGIA, CELLULARE E MOLECOLARE "L. CALIFANO," VIA S. PANSINI 5, 80131 NAPLES, ITALY.

fication of mutations at sites not recognized by restriction endonucleases (13). We altered the sequence of the sense primer such that amplification of DNA containing a G, but not any other nucleotide, at position 3116 of the IR gene, would generate a recognition site for the enzyme *Msp* I. This region was amplified as follows: 200 ng of genomic DNA from each patient were amplified in 50 μl of PCR buffer (10 mM Tris-HCl [pH 8.3], 1.5 mM $MgCl_2$, 50 mM KCl, 0.001% gelatin) containing 20 pmol of each oligonucleotide primer (nucleotides 3089 to 3115, sense strand; nucleotides 3191 to 3217, antisense strand) and 10 nmol of each deoxyribonucleotide triphosphate; one unit of thermus aquaticus DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) was added and 100 μl of mineral oil was layered over the samples. The samples were incubated at 95, 55, and 72°C, respectively, for 45, 50, and 50 s; this cycle was performed 36 times in a DNA-RNA amplifier (Biostar Violet, Rome, Italy). The amplified DNAs were restricted with *Msp* I and analyzed by electrophoresis in 2.5% agarose.

Because all the DNAs were digested by *Msp* I showing the presence of a G at position 3116 of the IR gene, we never detected Val^{996} allele in our diabetic patients.

Our data are consistent with a very low prevalence of the Val^{996} allele of the IR gene (<1/206 alleles) in the NIDDM population. Further studies are needed to establish the prevalence of other IR gene mutations among diabetic individuals.

References

1. Rosen OM: After insulin binds. *Science* 237:1452–58, 1988
2. Reaven GM: Banting Lecture: role of insulin resistance in human disease. *Diabetes* 37:1595–1607, 1988
3. Kadowaki T, Bevins CL, Cama A, Ojamaa K, Marcus-Samuels B, Kadowaki H, Beitz L, McKeon C, Taylor SI: Two mutant alleles of insulin receptor gene in a patient with extreme insulin resistance. *Science* 240:787–90, 1988
4. Yoshimasa Y, Seino S, Wihttaker J, Kakehi T, Kosaki A, Kuzuya H, Imura H, Bell GI, Steiner DF: Insulin-resistant diabetes due to a point mutation that prevents insulin proreceptor processing. *Science* 240:784–87, 1988
5. Kobayashi M, Sasaoka T, Takata Y, Ishibashi O, Sugibayashi M, Shigeta Y, Hisatomi A, Nakamura E, Tamaki M, Teraoka H: Insulin resistance by unprocessed insulin proreceptors point mutation at the cleavage site. *Biochem Biophys Res Commun* 153:657–63, 1988
6. Moller DE, Flier JS: Detection of an alteration in the insulin-receptor gene in a patient with insulin resistance, acanthosis nigricans, and the polycystic ovary syndrome (type A insulin resistance). *N Engl J Med* 319:1526–1529, 1988
7. Odawara M, Kadowaki T, Yamamoto R, Shibasaki Y, Tobe K, Accili D, Bevins C, Mikami Y, Matsuura M, Akanuma Y, Takaku F, Taylor SI, Kasuga M: Human diabetes associated with a mutation in the tyrosine kinase domain of the insulin receptor. *Science* 245:66–68, 1989
8. Taira M, Taira M, Hashimoto N, Shimada F, Suzuki Y, Kanatsuka A, Nakamura F, Ebina Y, Tatibana M, Makino H, Yoshida S: Human diabetes associated with a deletion of the tyrosine kinase domain of the insulin receptor. *Science* 245:63–66, 1989
9. Accili D, Frapier C, Mosthaf L, McKeon C, Elbein S, Permutt MA, Ramos E, Lander E, Ullrich A, Taylor SI: A mutation in the insulin receptor gene that impairs transport of the receptor to the plasma membrane and causes insulin resistant diabetes. *EMBO J* 8:2509–17, 1989
10. Klinkhamer MP, Groen NA, van der Zon GCM, Lindhout D, Sandkuyl LA, Krans HMJ, Moller W, Maassen JA: A leucine-to-proline mutation in the insulin receptor in a family with insulin resistance. *EMBO J* 8:2503–07, 1989
11. Magre J, Goldstein BJ, Kriauciunas KM, Kahn CR: Mutation of the insulin receptor at tyrosine-1146 associated with a novel tyrosine kinase defect in a patient with leprechaunism (Abstract). *Diabetes* 39:113A, 1990
12. Kadowaki T, Kadowaki H, Rechler MM, Serrano-Rios M, Roth J, Gorden P, Taylor SI: Five mutants alleles of the insulin receptor gene in patients with genetic forms of insulin resistance. *J Clin Invest* 86:254–64, 1990
13. Embury SH, Scharf SJ, Saiki RK, Gholson MA, Golbus M, Arnheim N, Erlich HA: Rapid prenatal diagnosis of sickle cell anemia by a new method of DNA analysis. *N Engl J Med* 12:656–61, 1987