cating that a translocation of transporters from cell interior to cell surface was induced; this effect was observed for both species of transporters. However, muscle transporters in PM were increased eightfold in response to insulin, compared with an ~1.5-fold increment for brain transporters. There was a differential effect of diabetes on the cellular content of each transporter protein. Muscle glucose transporters (per milligram of protein) were decreased by ~50% in both membrane subfractions from diabetic rats and were restored toward normal after insulin therapy. In contrast, brain transporters were not affected by diabetes or insulin treatment in any of the subfractions.

In a manner analogous to effects on cellular RNA, the amount of protein per cell in all membrane subfractions was reduced by 30 to 42% in diabetic rats (*5, 14*). Therefore, the actual reduction in the number of muscle transporters per cell was greater than that estimated by the protein blot analysis (Fig. 3a), in which equal amounts of protein were loaded in each lane. Also, insulin treatment of diabetic animals led to ~60% increase in cellular protein (*6, 14*), and therefore the protein blot analysis (Fig. 3a) represents underestimates of therapy-induced increments in muscle transporters per cell. Thus, the effects on muscle transporter mRNA correlated well with changes in the encoded protein, and pretranslational mechanisms may predominate in regulating the number of these glucose transporters in diabetes.

Karnieli *et al.* (*5*) found that total cellular glucose transporters are decreased by ~50% in adipocytes from streptozotocin-treated diabetic rats when measured by the cytochalasin B–binding assay (*3*), which identifies all species of glucose transporters. Our data now allow an estimate of the relative contribution of different species to overall transporter depletion. The selective diminution in the muscle transporter correlates well with the diabetes-induced depletion in cytochalasin B binding and may account for the major portion of transporter loss. Furthermore, changes in the amount of the muscle transporter may explain impaired insulin responsiveness in diabetic animals, suggesting that this transporter predominates in facilitating insulin-responsive glucose transport. These hypotheses are also supported by observations that cellular cytochalasin B–binding sites, insulin-stimulated glucose transport rates, and muscle glucose transporters are all increased after insulin therapy. It seems likely that brain glucose transporters mediate most of the basal glucose uptake, as both basal rates and brain transporters are not specifically regulated in these experimental animals.

Our data have implications regarding the pathogenesis of insulin resistance in individuals with diabetes mellitus. In type II (non-insulin-dependent) diabetes, the impaired ability of insulin to promote glucose uptake in target tissues is largely due to cellular depletion of glucose transporters (*1, 4*), and institution of therapy leads to partial reversal of insulin resistance, associated with enhanced activity of the glucose transport system (*1, 15*). Similarly, poorly controlled patients with type I (insulin-dependent) diabetes exhibit decreased insulin responsiveness for stimulation of glucose uptake that can be normalized after intensive insulin therapy (*16*). On the basis of our data, insulin resistance in diabetes and its amelioration with therapy could be due to changes in the amounts of muscle glucose transporter mRNA and protein contained in cells. Thus, decreased numbers of these transporters in target tissue may constitute a predominant biochemical lesion that maintains the diabetic state.

REFERENCES AND NOTES

1. W. T. Garvey, in *Pathogenesis of Non-Insulin-Dependent Diabetes Mellitus*, V. Grill and S. Efendic, Eds. (Raven, New York, 1988), pp. 171–200.
2. K. Suzuki and T. Kono, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2542 (1980).
3. S. W. Cushman and L. J. Wardzala, *J. Biol. Chem.* **255**, 4758 (1980); E. Karnieli *et al.*, *ibid.* **256**, 4772 (1981).
4. W. T. Garvey, T. P. Hueckstead, S. Matthaei, J. M. Olefsky, *J. Clin. Invest.* **81**, 1528 (1988).
5. E. Karnieli *et al.*, *ibid.* **68**, 811 (1981).
6. E. Karnieli, M. Armoni, P. Cohen, Y. Kanter, R. Rafaeloff, *Diabetes* **36**, 925 (1987); B. B. Kahn and S. W. Cushman, *J. Biol. Chem.* **262**, 5118 (1987).
7. M. Mueckler *et al.*, *Science* **229**, 941 (1985); H. K. Sarkar, B. Thorens, H. F. Lodish, H. R. Kaback, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5463 (1988).
8. M. J. Birnbaum, H. C. Haspel, O. M. Rosen, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5784 (1986).
9. H. Fukumoto *et al.*, *ibid.* **85**, 5434 (1988); B. Thorens *et al.*, *Cell* **55**, 281 (1988).
10. T. Kayano *et al.*, *J. Biol. Chem.* **263**, 15245 (1988).
11. M. J. Birnbaum, *Cell* **57**, 305 (1989).
12. M. M. Harpold *et al.*, *ibid.* **17**, 1025 (1979).
13. J. Vinten *et al.*, *Acta Physiol. Scand.*, in press. The monoclonal antibody to the erythrocyte transporter immunoprecipitates a protein in adipocyte membrane subfractions that exhibits glucose transport activity in reconstituted liposomes.
14. W. T. Garvey and T. P. Hueckstead, unpublished data.
15. W. T. Garvey *et al.*, *Diabetes* **33**, 346 (1985); T. P. Ciaraldi *et al.*, *ibid.* **31**, 1016 (1982).
16. R. R. Revers *et al.*, *J. Clin. Endocrinol. Metab.* **58**, 353 (1984); H. Yki-Jarvinen and V. A. Koivisto, *ibid.* **59**, 371 (1984).
17. W. T. Garvey *et al.*, *J. Biol. Chem.* **262**, 189 (1987).
18. J. W. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979).
19. C. Labarca and K. Paigen, *Anal. Biochem.* **102**, 344 (1980).
20. P. S. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201 (1980); E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975); A. Feinberg and B. Vogelstein, *Anal. Biochem.* **137**, 266 (1984).
21. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).

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Human Diabetes Associated with a Deletion of the Tyrosine Kinase Domain of the Insulin Receptor

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The insulin receptor has an intrinsic tyrosine kinase activity that is essential for signal transduction. A mutant insulin receptor gene lacking almost the entire kinase domain has been identified in an individual with type A insulin resistance and acanthosis nigricans. Insulin binding to the erythrocytes or cultured fibroblasts from this individual was normal. However receptor autophosphorylation and tyrosine kinase activity toward an exogenous substrate were reduced in partially purified insulin receptors from the proband's lymphocytes that had been transformed by Epstein-Barr virus. The insulin resistance associated with this mutated gene was inherited by the proband from her mother as an apparently autosomal dominant trait. Thus a deletion in one allele of the insulin receptor gene may be at least partly responsible for some instances of insulin-resistant diabetes.

TYROSINE-SPECIFIC PROTEIN KINASES participate in many cellular signal transduction events. The products of several oncogenes and certain growth factor receptors are tyrosine-specific protein kinases (*1*). The insulin receptor β subunit has an intrinsic tyrosine kinase activity that is activated by the binding of insulin to the

Table 1. Characteristics of the proband's family. Glucose (1.75 g per kilogram of body mass for the proband, sister-1, and sister-2 and 75 g for the mother and father) was ingested for oral glucose tolerance tests. Plasma glucose and serum insulin concentrations were measured by standard techniques after fasting for 14 hours and 2 hours after glucose ingestion. Insulin binding to erythrocytes (3×10^9 cells per milliliter) and fibroblasts (100 μg of protein) was measured as described (*4, 8, 21, 22*) and is expressed as a percentage of total counts after correcting for nonspecific binding.

Member	Age (years)	Body length (cm)	Body mass (kg)	Glucose tolerance test				Insulin binding (%)	
				Glucose (mg/dl)		Insulin (μU/ml)		Erythrocytes	Fibroblasts
				Fasting	2-hour	Fasting	2-hour		
Proband	17	136	36	127	361	55	123	6.1	0.93
Mother	40	140	33	238	368	100	110	8.4	1.2
Father	49	164	60	98	186	9.2	91	7.1	0.51
Sister-1	21	151	50	73	94	6.3	42		
Sister-2	19	156	50	74	74	9.2	21		
Normal range				<110	<120	<17	6.5–58	6.7–7.9	0.37–0.85

receptor α subunit (*2*). The tyrosine kinase activity of the insulin receptor is required for insulin signal transduction (*3–5*). Most instances of diabetes mellitus are caused by an insensitivity of target cells to insulin or by an impairment of insulin secretion, or both (*6*), and frequently the predisposition to develop diabetes is hereditary (*7*). It seems plausible that an abnormal structure of the tyrosine kinase domain of the insulin receptor could cause a decrease in receptor function, thereby contributing to insulin resistance in diabetes mellitus.

We have studied a 17-year-old Japanese female who exhibited insulin-resistant diabetes, short stature, and acanthosis nigricans. The mother of this individual shows the same phenotype, whereas the father and two siblings appear unaffected (Table 1). Erythrocytes and cultured fibroblasts from the proband and the mother had an insulin-binding capacity in the normal range (Table 1), but the cultured fibroblasts from both showed a below normal rate of 2-deoxyglucose uptake (*8*). Therefore, the insulin resistance in this instance seems to be due to a defect downstream from insulin binding. The extent of insulin-stimulated autophosphorylation of insulin receptors prepared from Epstein-Barr virus (EBV)–transformed lymphocytes of the proband was about half that seen with a nondiabetic control (Fig. 1A), and tyrosine kinase activity toward an exogenous substrate was also markedly reduced (Fig. 1B). Thus, it is possible that the defect of the insulin receptor kinase activity may be related to the cause of insulin resistance in the proband, as has also been suggested in other cases (*9, 10*).

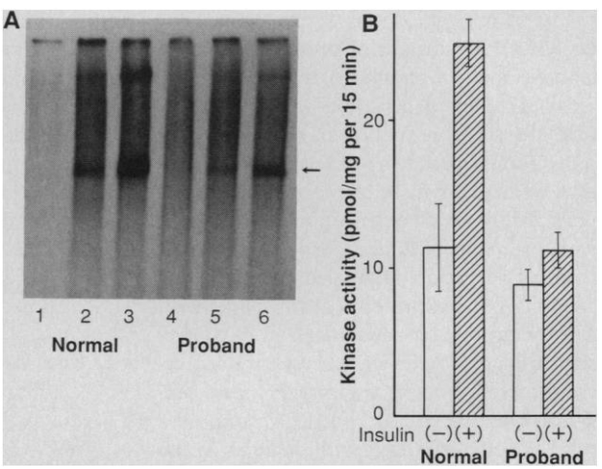

Fig. 1. (**A**) Autoradiogram showing the autophosphorylation of purified insulin receptors from a normal individual (lanes 1 to 3) and the proband (lanes 4 to 6). Lectin-purified insulin receptors were obtained from EBV-transformed lymphocytes as described (*23*). The numbers of insulin receptors in the preparations were equalized from insulin-binding data. The preparations were incubated without insulin (lanes 1 and 4) or with 0.1 n*M* (lanes 2 and 5) or 1 μ*M* insulin (lanes 3 and 6). Receptor phosphorylation was measured as described (*24*). The insulin receptors were immunoprecipitated with antibody to the insulin receptor (2G7) (*25*) and subjected to electrophoresis on SDS-polyacrylamide gels under reducing conditions. Arrow points to receptor β subunit. (**B**) Tyrosine kinase activity of purified insulin receptors from a normal individual and the proband. Receptors were purified and the receptor concentration was adjusted as in (A). The preparations were assayed with a synthetic copolymer containing glutamate and tyrosine (4:1) either in the absence or presence of 1 μ*M* insulin as described (*10*). The incorporation of ^{32}P into the polypeptide was measured in a liquid scintillation counter. The results represent the mean ± SD ($n = 3$).

To further examine the association of an insulin receptor defect with the insulin resistance of the proband, we analyzed restriction fragment length polymorphism of the insulin receptor gene in the family with insulin receptor cDNA (*11*) as a probe. Hybridization patterns of Bgl II–digested DNAs could be grouped into three allele types, A, B, and C (Fig. 2A). Allele C was found in the proband and the mother but not in nine other members of the family (Fig. 2B) or in 103 Japanese control subjects (*12*). The proband's maternal uncle and maternal grandfather were also said to be diabetic and had short stature (Fig. 2B). Therefore, insulin-resistant diabetes appeared to cosegregate with allele C and was probably transmitted as an autosomal dominant trait. Our data suggest that the abnormality of the insulin receptor gene of allele C may be at least partly responsible for the pathogenesis of this syndrome.

With the use of several region-specific insulin receptor cDNA probes, we determined that the 13-kb fragment (allele C) was derived from the 23-kb fragment (allele A) (Fig. 2A) (*13*). Thus, we characterized allele C by cloning the 13- and the 23-kb Bgl II fragments from the proband's DNA (Fig. 3A). A comparison of the restriction maps of the two fragments suggested that the mutation occurred downstream from the Bam HI site (Fig. 3A) of allele C. The nucleotide sequences of the Bam HI–Hind III fragment (400 bp) of the two clones and the Stu I–Bam HI fragment (350 bp) of allele A (which includes the next exon) were determined (Fig. 3B). A comparison of the two sequences shows that the mutation occurs at nucleotide 145, which is within the exon, just before the codon for Lys^{1030}. This amino acid is a part of the adenosine triphosphate (ATP) binding site of the receptor and is required for tyrosine kinase activity (*4, 5*). The exon containing the mutation corresponds to exon 17, which encodes the NH_2-terminal part of the kinase domain

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(*14*). The sequences of alleles A and C upstream from nucleotide 145 are identical whereas the downstream sequences are entirely different. By searching the Genebank database, we found that the new sequence of allele C was homologous to the consensus sequence of the Alu family (*15*), suggesting that the mutation resulted from recombination between exon 17 of the insulin receptor and an Alu sequence. Furthermore, DNA blot analysis showed that the exons downstream from exon 17 were not present in allele C (*16*). The coding sequence of this exon seems to continue to the stop codon at nucleotide 339 because nucleotide sequences similar to splicing donor and acceptor sites were not found in the downstream sequence from the junction point in allele C. Therefore, the putative product from the mutated gene probably has the new sequence of 65 amino acids at its COOH-terminus (Fig. 3B). This terminal amino acid sequence was not homologous to that of any other reported protein. Thus, the truncated receptor should not possess kinase activity.

We failed to detect the truncated insulin receptor on autoradiograms after immunoprecipitation of the proband's [^{35}S]methionine-labeled EBV-transformed lymphocytes with a monoclonal antibody to the human insulin receptor. This may have been due to the presence of proteins of similar molecular mass that were coprecipitated (*17*). However, truncated insulin receptors are apparently produced from the deleted gene because the insulin-binding capacity of the proband's cells was normal (Table 1). Cells of an individual who did not synthesize receptor protein from one allele showed half of the insulin-binding capacity of cells from a normal individual (*18, 19*). Previously, we have shown that mutated insulin receptors that did not exhibit insulin-activated kinase activity suppressed the function of native, normal insulin receptors in Chinese hamster cells that had been transfected with the mutated human insulin receptor cDNA (*4*). Similarly the truncated insulin receptors seem to suppress the function of the normal insulin receptors in cells from the proband (Fig. 1B).

Recently, two individuals that show insulin resistance and have abnormal insulin receptor genes have been described (*19, 20*); both individuals had point mutations in the α subunit domain of the receptor gene, and the mutations were transmitted as an autosomal recessive trait.

Individuals with diabetes mellitus, such as the one in our study, that are insulin-resistant and have disruptive mutations of the insulin receptor gene seem to be rare. However, more instances of noninsulin-dependent diabetes mellitus may be due to relatively minor mutations of the insulin receptor gene that cause slightly decreased affinity

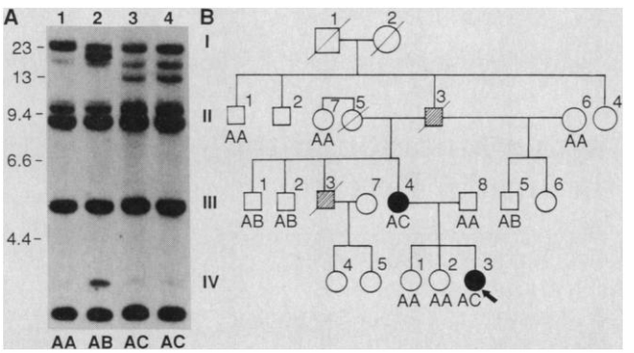
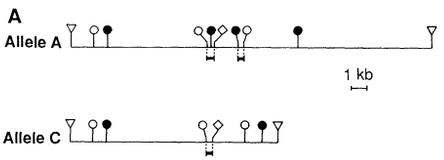


Fig. 2. (**A**) DNA blot of genomic DNA for linkage analysis. (Lane 1) Normal subject homozygous for a 23-kb fragment (allele A). (Lane 2) Normal subject heterozygous for 23- and 20-kb (allele B) fragments. (Lane 3) The proband and (lane 4) the mother, both heterozygous for 23- and 13-kb (allele C) fragments. A, B, and C under the autoradiogram indicate the alleles of the subjects and the size of the DNA fragments in kilobases is shown on the left. Genomic DNA (10 μg) from leukocytes was digested with Bgl II and separated by electrophoresis on a 0.7% agarose gel. The blot was incubated with ^{32}P-labeled full-length cDNA for the human insulin receptor under the conditions described (*26*) and then washed at 58°C in 15 m*M* NaCl, 1.5 m*M* sodium citrate, and 0.1% SDS. (**B**) Pedigree and linkage analysis of the proband's family. The alleles of the insulin receptor gene are shown below the symbols. Squares, male; circles, female; closed symbols, individuals affected with insulin-resistant diabetes and short stature; shaded symbols, individuals said to be affected with the same syndrome; arrow, the proband; slashed symbols, deceased. The proband's maternal grandfather (II-3) died in a severe diabetic state at age 45 years, despite undergoing insulin therapy. The proband's uncle (III-3) revealed an insulin-resistant state when undergoing an operation for a gastric ulcer at age 31 years and died without improvement of the diabetic state 5 days after the operation, despite the administration of a large amount of insulin. There is no evidence of consanguineous marriage in the family.



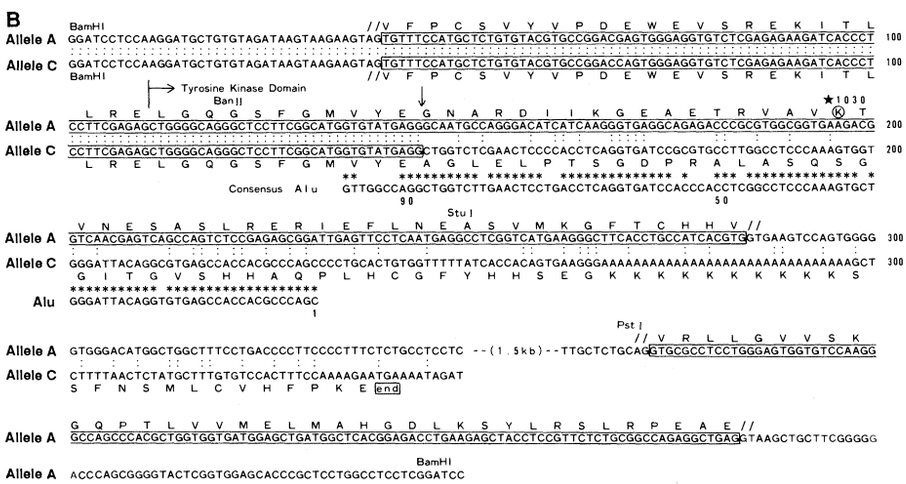


Fig. 3. (**A**) Comparison of restriction maps obtained from cloned DNA fragments of allele A and allele C from the proband. Allele A is the Bgl II 23-kb fragment and allele C is the Bgl II 13-kb fragment. Restriction sites are Bgl II (▽), Bam HI (○), Stu I (●), and Hind III (◇). Solid bars below the restriction maps [Bam HI–Hind III (0.4 kb) and Stu I–Bam HI (0.3 kb) fragments in allele A; Bam HI–Hind III fragment (0.4 kb) in allele C] denote regions used for DNA sequencing. (**B**) Nucleotide sequences across the mutation point in allele C and the corresponding regions of allele A of the proband. The Alu consensus sequence is also shown. Deduced amino acids in exons are denoted by the single letter code (*27*) above or below the nucleotide sequence. Colons (:) and asterisks (*) indicate positions at which the nucleotide sequence of allele A and allele C, and allele C and Alu, respectively, are identical. The position of Lys^{1030} is shown by (★). Arrow indicates the mutation point. The boxed sequences represent exons. High molecular weight genomic DNA was isolated from leukocytes of the proband and digested with Bgl II. The genomic library was constructed with phage vector EMBL3 by standard procedures and was screened with a region-specific cDNA probe [Rsa I–Rsa I fragment (nucleotide positions 3170 to 3737)] (*11*) that specifically hybridized to the 23- and 13-kb fragments. Two positive clones were isolated and characterized by restriction endonuclease digestion. The 13- and 23-kb Bgl II inserts were excised from the recombinant EMBL3 phage and subcloned into Bam HI–digested pUC118 or pUC119 vectors (Takara Shuzo). Sac I–Hind III fragments were subcloned into M13mp18 or M13mp19 vectors and sequenced by the dideoxy chain-termination method.

of the receptor for insulin or a slightly decreased kinase activity; in these cases, environmental factors such as obesity may trigger the onset of diabetes.

REFERENCES AND NOTES

1. T. Hunter, *Cell* **50**, 823 (1987).
2. M. Kasuga, Y. Zick, D. L. Blithe, M. Crettaz, C. R. Kahn, *Nature* **298**, 667 (1982).
3. D. O. Morgan, L. Ho, L. J. Korn, R. A. Roth, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 328 (1986).
4. Y. Ebina *et al.*, *ibid.* **84**, 704 (1987).
5. C. K. Chou *et al.*, *J. Biol. Chem.* **262**, 1842 (1987).
6. G. M. Reaven, *Diabetes Care 7* (suppl. 1), 17 (1984).
7. R. B. Tattersall and D. A. Pyke, *Lancet* **1972 ii**, 1120 (1972).
8. N. Hashimoto *et al.*, in preparation.
9. G. Grunberger, Y. Zick, P. Gorden, *Science* **223**, 932 (1984).
10. F. Grigorescu, J. S. Flier, C. R. Kahn, *J. Biol. Chem.* **259**, 15003 (1984).
11. Y. Ebina *et al.*, *Cell* **40**, 747 (1985).
12. Masato Taira *et al.*, unpublished data.
13. Masato Taira *et al.*, unpublished data.
14. S. Seino, M. Seino, S. Nishi, G. I. Bell, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 114 (1989).
15. P. L. Deininger, D. J. Jolly, C. M. Rubin, T. Friedmann, C. W. Schmid, *J. Mol. Biol.* **151**, 17 (1981).
16. Masato Taira *et al.*, unpublished data.
17. N. Hashimoto *et al.*, unpublished data.
18. F. Grigorescu *et al.*, *J. Clin. Endocrinol. Metab.* **64**, 549 (1987).
19. T. Kadowaki *et al.*, *Science* **240**, 787 (1988).
20. Y. Yoshimasa *et al.*, *ibid.*, p. 784.
21. K. K. Gambhir, J. A. Archer, L. Carter, *Clin. Chem.* **23**, 1590 (1977).
22. F. Nakamura *et al.*, *Endocrinol. Jpn.*, in press.
23. J. A. Hedo, L. C. Harrison, J. Roth, *Biochemistry* **20**, 3385 (1981).
24. F. Grigorescu, M. F. White, C. R. Kahn, *J. Biol. Chem.* **258**, 13708 (1983).
25. D. O. Morgan and R. A. Roth, *Biochemistry* **25**, 1364 (1986).
26. G. I. Bell, J. H. Karam, W. J. Rutter, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5759 (1981); E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
27. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln, R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Human Diabetes Associated with a Mutation in the Tyrosine Kinase Domain of the Insulin Receptor

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Insulin receptor complementary DNA has been cloned from an insulin-resistant individual whose receptors have impaired tyrosine protein kinase activity. One of this individual's alleles has a mutation in which valine is substituted for Gly^{996}, the third glycine in the conserved Gly-X-Gly-X-X-Gly motif in the putative binding site for adenosine triphosphate. Expression of the mutant receptor by transfection into Chinese hamster ovary cells confirmed that the mutation impairs tyrosine kinase activity.

MOST INDIVIDUALS WITH NONINSULIN-dependent diabetes mellitus (NIDDM) are resistant to the biological actions of insulin, and this is thought to be one of the primary factors giving rise to the disease (*1*). The binding of insulin to its receptor is the first step in insulin action, and defects in insulin receptor function may explain the insulin resistance in diabetic patients. When insulin binds to the extracellular domain of the receptor, the receptor undergoes autophosphorylation (*2*) and the tyrosine kinase associated with the intracellular domain (*3–5*) of the receptor is activated, thereby triggering the biological response within the target cell (*6, 7*). We have now identified a mutation in the insulin receptor gene of an individual with a form of NIDDM associated with severe insulin resistance.

The proband, a young Japanese male with the syndrome of insulin resistance and acanthosis nigricans (*8*), had a normal plasma glucose concentration when fasted but had the glucose tolerance typical of a diabetic. When the proband was fasted, his insulin level was high (150 μU/ml) and rose to >1000 μU/ml during an oral glucose tolerance test. Insulin binding to his circulating mononuclear cells was decreased, possibly due to down-regulation of insulin receptors. Although the number of insulin receptors on Epstein-Barr virus (EBV)–transformed lymphoblasts derived from the proband was at the lower limit of normal, there was a 50 to 80% decrease in receptor tyrosine kinase activity as measured with both solubilized insulin receptors and intact lymphoblasts (*8*).

To identify the molecular basis of the defect in the insulin receptor–associated tyrosine kinase activity, we cloned the cDNA encoding the proband's insulin receptor (Fig. 1). We determined the nucleotide sequence of four clones encoding the adenosine triphosphate (ATP) binding site of the

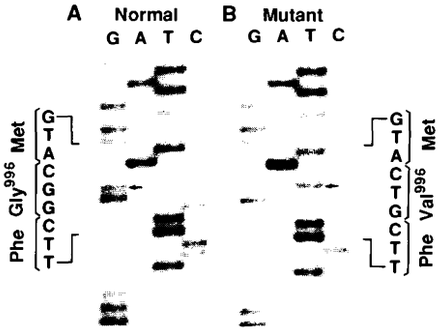

Fig. 1. Partial nucleotide sequence of two alleles of the proband's insulin receptor gene. A cDNA library was constructed in λgt10 from polyadenylated RNA that had been isolated from the proband's EBV-transformed lymphoblasts (*12, 18*). Approximately 2×10^6 recombinant bacteriophage were screened with an insulin receptor cDNA (*4*), and 44 positive clones were obtained. Fourteen clones encoding portions of the β subunit were recloned into pUC13, and both strands were sequenced by the dideoxy chain termination method with T7 DNA polymerase (Sequenase, United States Biochemical Corp.). (**A**) Clone 29 (nucleotides 2415 to 3705) had the same sequence as described (*5*) except at codon 1046 where an alternative histidine codon was present (CAT instead of CAC). Codon 996 (GGC) encoded glycine as in the normal sequence (*4, 5, 12*). The sequence of clone 29 was confirmed in another clone (clone 27, nucleotides 2963 to 3337). (**B**) Clone 8 (nucleotides 2743 to 4394) had the same sequence as described (*5*) except at codon 996, which was GTC instead of GGC, resulting in the substitution of valine for glycine at position 996. The sequence of clone 8 was confirmed in another clone (clone 25, nucleotides 3070 to 4405).

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