The human insulin-receptor (hINSR) gene spans a region of >120,000 base pairs (bp) on the short arm of chromosome 19. It is comprised of 22 exons or coding regions that vary in size from 36 to >2500 bp. To a large degree, the introns appear to divide the hINSR gene into segments that encode structural and/or functional elements of the hINSR protein. The exon-intron organization of the hINSR gene provides a clue to the evolutionary history of this gene and suggests that it is a mosaic constructed from protein-coding regions recruited from other genes. Eight mutations in the hINSR gene that result in expression of structurally abnormal proteins have been described. These mutations are associated with insulin resistance and provide insight into the role of the hINSR gene in the development of diabetes mellitus. Diabetes 39:129-33, 1990

Insulin exerts a wide variety of effects on responsive cells, resulting in increased glycogen, lipid, and protein synthesis (1-6). These effects include stimulation of glucose, amino acid and ion uptake, enhanced tyrosine and serine phosphorylation of cellular proteins, modulation of enzymatic activity of regulatory enzymes by dephosphorylation, and stimulation or repression of transcription of specific genes. In addition, insulin promotes cell growth. Although the cellular responses to insulin are diverse and in many instances tissue or cell specific, all are mediated by the insulin receptor (INSR).

The human INSR (hINSR) is an integral membrane protein present on the surface of all cells. The surface concentration of receptors varies widely from as few as 40 on circulating erythrocytes to >200,000 on adipocytes and hepatocytes. The mature hINSR is a heterotetramer of two α-subunits of 719 or 731 amino acids* and two 620-amino acid β-subunits (7,8). The insulin-binding α-subunit and the membrane-spanning protein tyrosine kinase β-subunit are generated by proteolytic processing of a common single-chain precursor. Cleavage of the proreceptor occurs during intracellular transfer from the endoplasmic reticulum to the plasma membrane. After cleavage of the proreceptor, the nascent α- and β-subunits are glycosylated on asparagine (N linked) and possibly serine/threonine residues (O linked). Thus, the α- and β-subunits have apparent M, of 135,000 and 95,000, respectively, which are much greater than those predicted from their sequences: the α- and β-subunits have predicted M, of 82,000 or 84,000 and 70,000, respectively. In the absence of the α-subunit, the endogenous tyrosine kinase of the β-subunit is constitutively active, suggesting that one function of the α-subunit is to repress the activity of the tyrosine kinase (9). Insulin binding to the α-subunit probably induces a conformational change in the receptor that relieves this repression.

The identification of genetic defects in the hINSR that are associated with extreme insulin resistance and glucose intolerance has provided new insight into the possible role of the hINSR in the development of non-insulin-dependent diabetes mellitus (NIDDM) (10-16). It is appropriate to consider the contribution of genetic variation in the hINSR gene in relation to the development of the common forms of NIDDM. Is there a subpopulation of diabetic patients who have mutations in the hINSR that impair its function but less severely than those associated with extreme insulin-resistant syndromes? We are in a position to address this important question.

*The numbering of the amino acid residues of the hINSR is for α-subunit and precursor of 731 and 1355 amino acids, respectively. The four basic amino acids at the proreceptor cleavage site are presumed to be removed from the COOH-terminal of the α-subunit during processing of the proreceptor.
EXON-INTRON ORGANIZATION OF hINSR GENE

The hINSR gene is located on the distal short arm of human chromosome 19 in the region of bands p13.3 → p13.2 (17). The low-density lipoprotein (LDL)-receptor gene is also in this region of chromosome 19 and is located toward the centromere and ~10–15 × 10^6 base pairs (bp) from the hINSR gene (18). Most of the hINSR gene has been isolated as a series of overlapping DNA fragments in the bacteriophage λ (19). These fragments span a region of >130,000 bp, which includes both the gene and flanking regions (Fig. 1). We have sequenced ~13,000 bp of the hINSR, including the 5′-flanking promoter region, each exon, and part of each intron (19, unpublished observations). The gene is composed of 22 exons and 21 introns. The exons range in size from 36 bp (exon 11) to >2500 bp (exon 22, most of which codes for the 3′-untranslated region of hINSR mRNA), whereas the 21 introns that separate the exons vary in size from ~500 to >25,000 bp (19). All of the introns interrupt protein-coding regions of the gene. The actual size of the hINSR gene is uncertain because parts of introns 2, 3, 9, and 10 were not isolated, but it must be >120,000 bp. Muller-Wieland et al. (20) isolated a fragment of the hINSR gene that contains both exons 1 and 2, and their data indicate that intron 1 is ~25,000 bp. The distribution of the exons is rather striking. Exons 1–11, which encode the α-subunit of the receptor, are dispersed over >90,000 bp. In contrast, exons 12–22, which encode the β-subunit, are located together in a region of ~30,000 bp. The promoter region of the hINSR gene has also been characterized, and its features are reviewed in Seino et al. (19).

The exon organization of the hINSR gene appears to reflect the structural organization of the protein, because many of the exons code for structural or functional modules of the protein (Fig. 1). Exons 1–3 code for the signal peptide, part of the insulin-binding region (21), and a very cysteine-rich domain of the protein that may also interact with insulin (22), respectively. The region encoded by exons 2–5 is also homologous to the corresponding region of the epidermal growth factor-receptor gene (19). Residues encoded by exon 6 appear to be located at the interface between adjacent α-subunits in the heterotetrameric form of the hINSR and contribute to the cooperative site-site binding interactions of the hINSR (23). Exon 11 is the smallest exon, only 36 bp in size, and alternative splicing of this exon results in the synthesis of hINSR proteins having α-subunits with different COOH-terminal sequences (7, 8, 24). Exon 12 codes for the tetrabasic amino acid sequence Arg-Lys-Arg-Arg, which is the site in the proreceptor molecule cleaved by the proteolytic processing enzyme, thereby generating the α- and β-subunits. Exon 12 also encodes the NH₂-terminal of the β-subunit; 20% of the amino acids in this region of the β-subunit are either serine or threonine, suggesting that the NH₂-terminal of the β-subunit may represent a region of O-linked oligosaccharide modification. Exon 15 codes for the membrane-spanning region of the β-subunit. Thus, exons 1–14 encode the extracellular region of the hINSR, whereas exons 16–22 encode the region that is localized within the cell. Exon 16 codes for a 22-amino acid segment that separates the transmembrane and tyrosine kinase domains. Exons 17–21 code for the protein tyrosine kinase; the amino acids involved in ATP binding by the kinase, residues 1003–1008 (Gly-Gln-Gly-Ser-Phe-Gly) and 1030 (Lys), are all encoded by exon 17. The tyrosine residues that are autophosphorylated by the kinase on insulin binding are encoded by exons 20 (Tyr 1158, 1162, and 1163) and 22 (Tyr 1328 and 1334). Exon 22 also codes for the highly charged COOH-terminal of the β-subunit. The data suggest that exons encode functional/structural domains of the hINSR protein and that the hINSR gene, like the LDL-receptor gene (25, 26), is a mosaic constructed from exons recruited from other sources.

ALTERNATIVE SPLICING GENERATES AMINO ACID-SEQUENCE ISOFORMS OF hINSR

The α- and β-subunits are generated by proteolytic processing of a precursor of 1370 or 1382 amino acids, which includes a 27-amino acid signal peptide in addition to the 1343 or 1355 proreceptor molecule (7, 8). The difference in size of the α-subunit and its precursors is due to tissue-specific, possibly developmentally regulated, alternative splicing of exon 11, which codes for a 12-amino acid segment at the COOH-terminal of the α-subunit (24). Brain and

FIG. 1. Map of human insulin-receptor (hINSR) gene. Relative locations and sizes of exons (■) and introns are indicated. Oblique lines, regions of introns 2, 3, 9, and 11 that have not been cloned. Location of mutations in hINSR gene are indicated: O, missense; □, nonsense; –, deletion. Table 1 describes mutations in greater detail. Domains of hINSR protein encoded by each exon or group of exons are below gene map. kb, Kilobase.
spleen express almost exclusively the 719-amino acid α-subunit, whereas other tissues, including placenta, liver, kidney, and adipose tissue, express α-subunits of both 719 and 731 amino acids. It is unknown whether both types of α-subunit are expressed in an individual cell, thereby generating a mixed population of receptors, including proteins having two α-subunits of either 719 or 731 amino acids and hybrid molecules with one α-subunit of each type. However, because all cultured cell lines that have been examined express only a single type of α-subunit, we suspect that individual cells express only one form of α-subunit, i.e., either 719 or 731 amino acids. The biological consequences of this diversity of hINSR isoforms is unknown. Transfection of cultured cells with cloned genes encoding recombinant hINSR protein representing both hINSR isoforms has indicated that each is biologically active and capable of binding insulin, stimulating autophosphorylation, and mediating postreceptor responses (27–29). However, preliminary studies suggest that the hINSR isoform with an α-subunit of 731 amino acids may have reduced affinity for insulin (30). Additional studies of the splicing of hINSR mRNA might account for some of the observed heterogeneity in size of the protein expressed in different tissues.

**GENETIC VARIATION IN hINSR GENE**

Genetic disorders of extreme hormone resistance provide a unique opportunity to examine the functional consequences of expression of an abnormal receptor. Extreme insulin resistance is associated with three syndromes: the type A syndrome of insulin resistance and acanthosis nigricans, leprechaunism, and the Rabson-Mendenhall syndrome (31). Recent studies have identified eight different mutations in one or both of the INSR alleles of individuals with these syndromes (Fig. 1; Table 1). These mutations affect both the biosynthesis of the receptor and its biochemical properties and include four mutations in the α-subunit (1 patient is a compound heterozygote and has different mutations in each of the parental INSR alleles; 10–12), one at the proreceptor processing site (13), and three in the β-subunit (14–16). Two of the α-subunit mutations (Pro239 and Val220) appear to alter the structure of the protein such that posttranslational processing and transport of the receptor to the plasma membrane is delayed, thereby resulting in reduced numbers of receptors on the cell surface (11,12). Individuals expressing both a normal allele and the Pro239 mutation have mild insulin resistance.

Subject 3 is a compound heterozygote expressing two different α-subunit mutations (10, Table 1). There is a nonsense mutation in the codon for amino acid 672 of the paternal derived allele, which results in the synthesis of a truncated 671-amino acid fragment of the α-subunit; such a molecule would be expected to be secreted from the cell and to be biologically inactive. This patient's maternally derived hINSR allele has a missense mutation (Glu462) that results in the expression of a protein with qualitative abnormalities in insulin binding, including increased stability of the insulin-hINSR complex. Such a mutation might impair the release of insulin in the acidic endosome after internalization of the insulin-hINSR complex and thereby affect hINSR recycling. Cells from the patient's mother, which express both normal and mutant (Glu462) INSRs, also exhibited qualitative abnormalities in insulin binding similar to the patient; however, the mother was neither insulin resistant nor diabetic. By contrast, the father, who expresses both the normal INSR protein and the truncated molecule, was moderately insulin resistant with impaired glucose tolerance, although not overtly diabetic. It is unclear why the Glu462 allele in the patient is less effective than a normal allele in complementing the nonsense mutation.

A mutation at the proreceptor processing site (Ser725) results in the expression of fully glycosylated but uncleaved proreceptors on the cell surface (13). This mutant INSR is capable of binding insulin and undergoing insulin-stimulated autophosphorylation, albeit weakly and at high insulin concentrations, and provides an elegant demonstration that the hINSR acquires normal insulin affinity and sensitivity only after proteolytic separation of the α- and β-subunits.

Three mutations in the β-subunit that represent postbinding defects in insulin action have been described, including two missense mutations (Val1006 and Ser220), 14,15 and a deletion involving exons 17–22 that results in the expression of a receptor lacking the tyrosine kinase domain (16). All are

**TABLE 1**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mutation</th>
<th>Exon</th>
<th>Ethnic origin</th>
<th>Biochemical properties of mutant protein</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Subunit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Leu 233 → Pro</td>
<td>3</td>
<td>Dutch</td>
<td>Delayed transport to cell surface</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Phe 382 → Val</td>
<td>5</td>
<td>Venezuelan</td>
<td>Delayed transport to cell surface</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Lys 460 → Glu</td>
<td>6</td>
<td>American</td>
<td>Qualitative abnormalities in insulin binding</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Gin 672 → AM</td>
<td>10</td>
<td></td>
<td>Truncated α-subunit</td>
<td></td>
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<tr>
<td>Proreceptor processing site</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Arg 735 → Ser</td>
<td>12</td>
<td>Japanese</td>
<td>Impaired proreceptor processing</td>
<td>13</td>
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<tr>
<td>β-Subunit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Gly 1008 → Val</td>
<td>17</td>
<td>Japanese</td>
<td>Decreased tyrosine kinase activity</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>Trp 1200 → Ser</td>
<td>21</td>
<td>American</td>
<td>Decreased receptor affinity and autophosphorylation</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>&gt;10-kb deletion</td>
<td>17–22</td>
<td>Japanese</td>
<td>Decreased tyrosine kinase activity</td>
<td>16</td>
</tr>
</tbody>
</table>

Subjects 1, 2, and 4 are homozygous for indicated mutation. Subject 3 is a compound heterozygote having inherited different mutations from each parent. Subjects 5–7 are heterozygous and express both mutant and normal insulin-receptor proteins. AM, amber (TAG) translation termination codon; kb, Kilobase.
associated with decreased or defective tyrosine kinase activity or autophosphorylation. The mutation at residue 1008 is of a conserved glycine residue that is part of the ATP binding site of all protein kinases. The codominant expression of normal INSRs and receptors having mutations in the tyrosine kinase domain appears to result in suppression of the function of the normal protein. The dominant negative character of these mutations may be due to the fact that the hINSR is a heterotetramer and that heterotetramers formed from two mutant subunits and mixed heterotetramers having one normal and one mutant subunit are inactive or have reduced activity. As a consequence, only 25% of the receptors would possess full biological activity.

In addition to mutations in the hINSR gene that are associated with insulin resistance, population-association studies suggest that there is also genetic variation in the region of the hINSR gene that increases (32) or decreases (33) the risk for NIDDM. The increased risk could be due to the expression of abnormal receptors that suppress the function of the normal receptors as suggested above. The decreased risk associated with some hINSR haplotypes might be due to genetic variation that increases expression of the hINSR and results in increased numbers of receptors per cell.

Along with nucleotide substitutions that result in the expression of an abnormal protein, silent substitutions in the coding region of the hINSR gene that alter the sequence of the gene and mRNA but not the protein have been described (8, 10, 19). Several restriction-fragment–length polymorphisms have also been reported at the hINSR locus (33–42), and all appear to be located within introns.

CONCLUSION

The causes of NIDDM are unclear. Although impaired β-cell function and insulin resistance are both features of NIDDM, controversy exists as to which defect is primary. Studies of patients expressing abnormal insulin (43–45) or INSR proteins (10–16) have indicated that mutations in either of these genes can be associated with NIDDM. In fact, insulin gene mutations represent a paradigm for β-cell defects that can cause diabetes. Similarly, INSR mutations are an example of a defect in a responsive cell that can contribute to the development of this disorder.

In contrast to insulin gene mutations, which are extremely rare, mutations in the hINSR gene may be more common. Moreover, because heterozygous individuals who express both normal and mutant receptors may exhibit mild insulin resistance, e.g., parents of subjects 1 and 3 (Table 1; 10, 12), it seems reasonable to consider that codominant expression of normal and abnormal hINSR proteins may contribute to the development of NIDDM. Although linkage studies of 20 American Black families in which at least two siblings had NIDDM suggested that the INSR gene was not the major susceptibility locus for NIDDM, at least in this population (46), the INSR gene may still contribute to the development of NIDDM in a small but measurable subpopulation of patients. The isolation and characterization of the hINSR gene and recent technical developments provide a strategy for examining the contribution of this gene to the development of NIDDM by amplifying individual exons of the hINSR gene with the polymerase chain reaction (PCR) and then sequencing the amplified DNA. Where should we look for hINSR mutations? Because mutations in the tyrosine kinase domain of the hINSR are associated with severe insulin resistance in the heterozygous state, whereas heterozygosity for mutations in the α-subunit appears to be associated with less-severe insulin resistance, the NIDDM subjects should first be classified as to the degree of insulin resistance. In the most-resistant subjects, PCR amplification and sequencing of exons 17–21, which code for the tyrosine kinase domain of the hINSR, should be the first priority. In the less-resistant patients, the analysis should begin with the amplification and sequencing of exons 2 and 3, which appear to be involved in insulin binding. Examination of the sequences of selected regions of the hINSR gene in 50–100 well-characterized NIDDM subjects could provide valuable insight into the contribution of this gene to the development of this disorder.

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