Mutations in the Insulin Receptor Gene in Patients with Genetic Syndromes of Insulin Resistance and Acanthosis Nigricans

Domenico Accili, Fabrizio Barbetti, Alessandro Cama, Hiroko Kadowaki, Takashi Kadowaki, Eiichi Imano, Rachel Levy-Toledano, and Simeon I. Taylor

Diabetes Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

Mutations of the insulin receptor gene have been identified in patients with genetic syndromes of insulin resistance associated with acanthosis nigricans. These mutations impair insulin responses by reducing the number of insulin receptors on the surface of target cells, or by reducing the receptor's ability to bind insulin or to undergo insulin-stimulated auto-phosphorylation, an important step in insulin action. Studies of mutant receptors expressed in transfection systems have contributed to our understanding of the structure-function relationships of the insulin receptor. J Invest Dermatol 98:77S–81S, 1992

Insulin resistance contributes importantly to the pathogenesis of noninsulin-dependent diabetes mellitus (NIDDM) [1,2]. We have focused our studies on genetic syndromes of insulin resistance. Because of the central role of the insulin receptor in mediating insulin action [3], we have begun by examining the insulin receptor gene in insulin-resistant patients. We have selected patients who manifest an extreme degree of insulin resistance in the hope that the severe insulin resistance would be associated with major biochemical defects, thereby simplifying the task of identifying the molecular defect. In this review, we will summarize the mutations that have been identified in the insulin receptor genes of patients with extreme insulin resistance. Multiple different types of mutations have been identified [3]. In addition to elucidating the molecular genetics of human insulin resistance, these studies have begun to give new insights into the structure-function relationships of the insulin receptor protein.

GENETIC SYNDROMES OF INSULIN RESISTANCE

Patients with inborn errors in the pathways of insulin action manifest an extreme degree of resistance to the biologic actions of insulin. However, many of these patients are not diabetic. In some patients, the levels of insulin rise to the point at which they are sufficiently high (often 10 to 100 times above the normal range) to maintain relatively normal glucose tolerance. Nevertheless, some patients—especially the patients with the most severe degree of insulin resistance—develop fasting hyperglycemia and overt diabetes. Two mechanisms contribute to the development of hyperinsulinemia in insulin-resistant patients. First, as reflected by the increase in the levels of C-peptide in plasma, the beta cell increases the rate of insulin secretion. Second, because receptor-mediated endocytosis is the principal route by which insulin is cleared from plasma, a decrease in the number of insulin receptors on the cell surface decreases the insulin clearance rate [4]. Two clinical features are commonly observed in patients with all of the syndromes of extreme insulin resistance, irrespective of the biochemical mechanism that causes the insulin resistance:

1. Acanthosis Nigricans: This skin lesion is characteristically associated with extreme insulin resistance (Fig 1). It tends to correlate with hyperinsulinemia. In patients with insulin resistance caused by anti-receptor autoantibodies (type B extreme insulin resistance), acanthosis nigricans waxes and wanes in association with the appearance and disappearance of the insulin resistance. This has led to the hypothesis that acanthosis nigricans may be caused by a "toxic" effect of hyperinsulinemia upon the skin. It has been postulated that insulin at high concentrations may activate IGF-1 receptors through a mechanism of "specificity spillover." IGF-1 receptors would in turn mediate proliferative effects on epidermal cells [5].

2. Hyperandrogenism: Levels of plasma testosterone are commonly elevated in premenopausal women with extreme insulin resistance [6–8]. The elevated levels of testosterone result from overproduction of testosterone by the ovaries. As with acanthosis nigricans, the elevated levels of testosterone correlate with hyperinsulinemia. Clinically, the elevated levels of testosterone are manifested as a syndrome of polycystic ovaries, oligomenorrhea, and hirsutism.

Whereas all of the syndromes of extreme insulin resistance share some features in common, multiple distinct syndromes can be defined based upon the presence or absence of specific clinical features [3]. For example, type A extreme insulin resistance is defined by the triad of insulin resistance, acanthosis nigricans, and hyperandrogenism in the absence of obesity or lipoatrophy [8]. In lipoatrophic diabetes, there is atrophy of subcutaneous fat, hypertriglyceridemia, and fatty metamorphosis of liver [9]. Patients with leprechaunism have multiple abnormal features, including intratuberine growth retardation and fasting hypoglycemia [10]. The Rabson-Mendenhall syndrome is associated with abnormalities of teeth and nails and, reportedly, pineal hyperplasia [11,12].

SUBUNIT STRUCTURE OF THE INSULIN RECEPTOR

Insulin exerts its biologic actions through a cell surface receptor. The receptor is an oligomeric glycoprotein that consists of two α-and two β-subunits that are assembled into a heterotetrameric structure (Fig 2). The receptor is encoded by a single gene located on the

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Reprint requests to: Dr. Domenico Accili, Diabetes Branch, NIDDK, Bethesda, MD 20892.

Abbreviations:

mRNA: messenger RNA
NIDDM: noninsulin-dependent diabetes mellitus
short arm of chromosome 19 [13]. The α and β subunits are produced by proteolytic cleavage of a single high-molecular-weight precursor molecule (M₀ ≈ 190,000). The α subunit (M₀ ≈ 135,000) is entirely extracellular and provides the insulin binding site. The β subunit (M₀ ≈ 95,000) is a transmembrane subunit that contains a single transmembrane domain [15,16]. The β subunit anchors the α subunit to the plasma membrane. In addition, the intracellular portion of the β subunit contains the tyrosine kinase domain of the receptor. When insulin binds to the extracellular domain of the receptor, this activates autophosphorylation of tyrosine residues in the β subunit by the receptor tyrosine kinase [17–19]. Receptor autophosphorylation activates the receptor to phosphorylate other intracellular proteins. There is abundant evidence suggesting that activation of the receptor tyrosine kinase is necessary for the receptor to mediate insulin action [20–23].

The receptor precursor undergoes multiple post-translational processing steps within the endoplasmic reticulum and Golgi apparatus, including proteolytic cleavage into α and β subunits. N-linked glycosylation, O-linked glycosylation, and acylation [24]. The mature receptor is transported to the cell surface where it is inserted into the plasma membrane. Insulin binds to the receptor on the cell surface, thereby activating the receptor tyrosine kinase. In addition, subsequent to insulin binding, the insulin/insulin receptor complex undergoes receptor-mediated endocytosis. Once within the cell, the receptor partitions between two alternate fates: recycling back to the cell surface for reutilization or intracellular degradation (possibly, within lysosomes). Clearly, this is an extremely complicated pathway. Defects in any one of these steps can impair insulin receptor function and cause insulin resistance. In the remainder of the chapter, we will review the various types of mutations that have been identified in the insulin receptor gene, and classify them according to the molecular mechanisms by which they impair the function of the insulin receptor.

MUTATIONS IN THE INSULIN RECEPTOR GENE

It is possible to classify mutations in the insulin receptor gene into at least five classes (Table I) [3], based upon the molecular mechanism whereby the mutation impairs the function of the receptor. (Note that some mutations may cause multiple defects in receptor function so that some mutations are classified in more than one class.)

Class 1: Decreased Rate of Receptor Biosynthesis

Premature Chain Termination Mutations: Several mutations have been identified that impair receptor biosynthesis. Four nonsense mutations have been identified: at codons 133, 672, 897, and 1000 [25–27]. In addition, two deletion mutations have been identified that result in premature chain termination mutations: i) deletion of exon 14 that causes a frame shift and the introduction of an in-frame chain termination codon [42]; and ii) deletion of the entire gene downstream from codon 1012 in exon 17 resulting in a fusion protein with a premature chain termination [43]. Some of these mutations—the deletion of exon 14 and also the nonsense mutations at codons 133, 672, and 897—truncate the receptor upstream from the transmembrane domain. Accordingly, these truncated receptors appear not to be expressed on the cell surface. Furthermore, three of the nonsense mutations—at codons 133, 897, and 1000—
have been demonstrated to cause an 80–90% decrease in the level of insulin receptor mRNA [26,27]. This reduction in the level of mRNA is predicted to decrease the rate of receptor biosynthesis. Thus, mutations that result in premature termination of translation impair receptor function by one or more of the following mechanisms: i) reduction of the number of insulin receptors by reducing the level of mRNA [26,27]; ii) truncation of the receptor upstream from the transmembrane domain so that the receptor is not expressed on the cell surface [25,26,42]; or iii) deletion of important functional domains in the intracellular portion of the receptor [27,43].

**Other Cis-Acting Mutations that Decrease Levels of mRNA:** Many different types of mutations are known to decrease levels of mRNA without altering the coding sequence of the gene. Although none of these mutations have been explicitly identified in the insulin receptor gene, there is strong evidence suggesting the existence of this type of mutation. We have investigated a patient with leprechaunism (leprechaun/Minn-1) whose cells have a marked decrease in the level of insulin receptor mRNA [26,44]. In the allele inherited from the father, there is a nonsense mutation at codon 897 that acts in a cis-dominant fashion to decrease mRNA. However, the allele inherited from the mother is also underexpressed even though the coding sequences of all 22 exons of the gene are normal. Nevertheless, studies of the expression of this allele in cells from the patient’s mother provided compelling evidence that there is a mutation in this allele.

**Class 2: Impaired Transport of Receptors to the Cell Surface** Patients A-5 and A-8 are two sisters who are members of a consanguineous kindred in which the parents are first cousins [28]. Both sisters with type A extreme insulin resistance are homozygous for a mutation substituting valine for phenylalanine at position 382 in the α-subunit of the insulin receptor [28]. Expression studies demonstrated that the Val382 mutation impairs transport of the receptor through the endoplasmic reticulum and Golgi with a consequent decrease in the number of receptors expressed on the cell surface [28]. We have identified two additional mutations that impair intracellular transport of receptors through the endoplasmic reticulum and Golgi with consequent decrease in the number of receptors expressed on the cell surface [28]. We have identified two additional mutations that impair intracellular transport of receptors through the endoplasmic reticulum and Golgi with a consequent decrease in the number of receptors expressed on the cell surface [28].

**Class 3: Decreased Affinity of Insulin Binding** In addition to the Lys15 mutation, another mutation (i.e., serine for Arg) has been reported to decrease the affinity of insulin binding. Arg795 is the fourth amino acid in an Arg-Lys-Arg-Arg sequence that separates the α subunit from the β subunit in the insulin receptor precursor. The Ser795 mutation inhibits the cleavage of the precursor into two separate subunits [30–32] and decreases the affinity with which the receptor binds insulin.

**Class 4: Defects in Receptor Tyrosine Kinase Activity** At least five missense mutations have been identified in the intracellular domain of β-subunit of the insulin receptor: Gly1008 → Val [34], Met1153 → Ile [38], Ala1134 → Thr [37], Ala1155 → Glu [39], Trp1208 → Ser [35,36]. The Val1008 mutation was identified in a young man with insulin resistance and acanthosis nigricans [34]; the remainder of the mutations were identified in young women with the syndrome of hyperandrogenism, insulin resistance, and acanthosis nigricans. Gly1008 is the third glycine residue in the highly conserved Gly1006-X-Gly1003-X-Gly1008-Y-Lys1009 motif that provides part of the binding site for ATP, the phosphate donor for the tyrosine kinase reaction. Thus it is likely that the Val1008 mutation impairs tyrosine kinase activity because it distorts the ATP binding site in the tyrosine kinase domain. The other four mutations alter amino acid residues (Met1153, Ala1134, Ala1154, and Trp1208) that are highly conserved in the amino acid sequences of tyrosine kinases in the insulin receptor family although their precise roles in enzyme structure and function are not understood.

**What is the mechanism whereby these mutations cause the phenotype of insulin resistance in a dominant fashion? Although this question has not been answered with certainty, the leading hypothesis relates to the oligomeric structure of the receptor. If mutant insulin receptor heterodimers (αβn) and wild type insulin receptor heterodimers (αβm) associate with one another randomly and with equal affinity, then three different heterotetramers would form in a ratio of 1:2:1 — αβmβm, αβnβm, and αβnβn. If the hybrid heterotetramer (αβnβm) were impaired in its tyrosine kinase activity, then a mutation in a single allele might lead to a 75% reduction in insulin receptor tyrosine kinase activity.

**Class 5: Accelerated Degradation of the Insulin Receptor** A mutation substituting glutamic acid for Lys460 was identified in a patient with leprechaunism (leprechaun/Ark-1) [25]. This mutation, inherited from the patient’s mother, is recessive in that the mother has normal glucose tolerance and does not appear to be insulin resistant. With normal insulin receptors (Lys460), decreasing the pH from 7.8 to 6.0 causes a tenfold acceleration in the rate at which [125I]insulin dissociates from its receptor. The effect of acid pH to accelerate [125I]insulin dissociation is markedly blunted with the Glu460-mutant receptor. We have obtained evidence that the Glu460 mutation causes insulin resistance by accelerating the rate of receptor degradation [40]. According to this hypothesis, the cause of insulin resistance is a decrease in the number of insulin receptors on the surface of target cells that results from an accelerated rate of receptor degradation.

A similar defect in pH sensitivity of insulin binding was observed with another mutant receptor in which serine was substituted for Asn462 [27]. It is intriguing that the Glu460 and Ser462 mutations map within two amino acid residues of one another.

**GENETICS OF INSULIN RESISTANCE** Many of the patients with genetic forms of extreme insulin resistance have mutations in the insulin receptor gene [3,25–43]. Nevertheless, there may be genetic heterogeneity within these syndromes. The degree of insulin resistance is extremely variable among these patients. For example, some patients are so severely insulin resistant that they have fasting hyperglycemia and overt diabetes despite having fasting plasma insulin levels > 100 μU/ml (normal, ≤ 20 μU/ml). This type of patient may require several thousand units of insulin per day to achieve acceptable control of the level of glucose in the plasma. There are relatively few patients with such severe insulin resistance. We have investigated several patients in this category, all of whom have had two mutant alleles of the insulin receptor gene (Table II). For example, two sisters (patients A-5 and A-8) were members of a consanguineous kindred and were homozygous for a missense mutation in the insulin receptor gene [28]. Another patient (patient A-1) was a compound heterozygote,
Table II. Patients with Mutations in the Insulin Receptor Gene†

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>A-1</td>
<td>Amber13/Ser62</td>
<td>[27]</td>
</tr>
<tr>
<td>A-5 and A-8</td>
<td>Val182 (homozygous)</td>
<td>28</td>
</tr>
<tr>
<td>Bl-1</td>
<td>Thr1134/WT</td>
<td>37</td>
</tr>
<tr>
<td>A-3</td>
<td>Glu1355?</td>
<td>39</td>
</tr>
<tr>
<td>A-6</td>
<td>Ile1239?</td>
<td>38</td>
</tr>
<tr>
<td>Bl-2</td>
<td>Ser1986/WT</td>
<td>[35,36]</td>
</tr>
<tr>
<td>Chiba-1</td>
<td>ΔExon17/WT</td>
<td>43</td>
</tr>
<tr>
<td>Chiba-2</td>
<td>ΔExon14?</td>
<td>42</td>
</tr>
<tr>
<td>Kyushu-1 and -2</td>
<td>Ser259 (homozygous)</td>
<td>[29,30]</td>
</tr>
<tr>
<td>Sapporo-1</td>
<td>Val1008?</td>
<td>27</td>
</tr>
</tbody>
</table>

| Rabson-Mendenhall syndrome | RM-1 | Lys37/Opal1000 | [27] |

| Leprechaunism | Ark-1 | Glu460/Amber672 | 25 |
| Gendemarslen | Pro208 (homozygous) | 41 |
| Minn-1 | Opal897/Unidentified | 26 |
| Winnipeg | Arg209 (homozygous) | [27] |

† Listed are the mutations in the insulin receptor gene that have been identified in patients with genetic forms of insulin resistance. WT refers to a wild type allele that encodes a receptor with a normal amino acid sequence. A question mark refers to an allele that is thought to be normal, but where the nucleotide sequence has been reported for only a portion of the protein coding domain. Amber and opal refer to nonsense mutations corresponding to codons UAG and UGA, respectively. Deletion mutations are abbreviated as ΔExon17/WT (22) (a deletion beginning after codon 1012 in the 3' part of exon 17 and extending downstream through exons 18-22) and ΔExon14 (a deletion of exon 14).

**CORRELATION OF CLINICAL SYNDROME WITH GENOTYPE**

Mutations have been identified in the insulin receptor gene in patients with several distinct syndromes: leprechaunism, the Rabson-Mendenhall syndrome, and type A insulin resistance. What determines which syndrome a patient will develop? Because the clinical syndromes do not correlate with the type of mutation, it seems most likely that it is the severity of insulin resistance that determines the clinical manifestations. For example, patients with leprechaunism appear to have the most extreme degree of insulin resistance. All of the patients with leprechaunism have had two mutant alleles of the insulin receptor gene; two were compound heterozygotes [25,26] and two were homozygotes [27,41]. Some patients with type A extreme insulin resistance have also been reported to have two mutant alleles: patient A-1, who is a compound heterozygote, as well as two sisters in each of two consanguineous pedigrees, all of whom were homozygous for mutations in the insulin receptor gene [28,30-32]. However, some patients with type A extreme insulin resistance have been reported to be heterozygous for a single mutant allele of the insulin receptor gene [34-43]. As exemplified by the comparison between leprechaun/Ark-1 and her father [25], the degree of insulin resistance observed in heterozygotes is less severe than the insulin resistance in homozygotes and compound heterozygotes. The father of leprechaun/Ark-1 is heterozygous for a nonsense mutation in the insulin receptor gene whereas his other allele encodes a normal receptor. The presence of a second mutant allele in the daughter is the cause not only of her more severe degree of insulin resistance but also the multiple phenotypic abnormalities associated with the syndrome of leprechaunism. Similarly, the mother of leprechaun/Winnipeg—an obligate heterozygote for the Arg209 mutation—is insulin resistant and hyperinsulinemic [10]. Thus, although the phenotype of leprechaunism is recessive, the phenotype of insulin resistance caused by the Arg209 mutation is inherited in a co-dominant fashion.

The mechanism by which insulin elicits its multiple biologic responses in target cells is extremely complex. For target cells to respond to insulin, this requires the function of many proteins encoded by many genes. At least in theory, each of these genes is a candidate to be the locus of a mutation causing insulin resistance in NIDDM. With the development of new molecular scanning techniques, it has become easier to identify mutations in candidate genes, and to isolate disease-linked genes (Fig 3). As progress is made in the identification and cloning of the many genes that allow for the normal response to insulin, it seems likely that it will be possible to identify the genes that are targets of mutations that cause insulin resistance in NIDDM.

**REFERENCES**

7. Barbieri RL, Ryan KJ: Hyperandrogenism, insulin resistance, and acan-