An Arg for Gly Substitution at Position 31 in the Insulin Receptor, Linked to Insulin Resistance, Inhibits Receptor Processing and Transport*

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In a patient with Leprechaunism, we have characterized a new mutation in the insulin receptor substituting Arg for Gly at position 31. The proband, the mother, and the maternal grandfather were heterozygous for the mutation. Fibroblasts of the proband show a strongly reduced number of high affinity insulin receptors on the cell surface, whereas fibroblasts of the healthy mother and grandfather show moderately reduced insulin receptor numbers. In the other family members neither the binding defect nor the Arg31 mutation was found.

The Arg31-mutant receptor was overexpressed in Chinese hamster ovary cells. In these cells the mutant αβ-proreceptor was not proteolytically cleaved and no transport to the cell surface took place. The proreceptor was unable to bind insulin and to undergo autophosphorylation. In addition, the proreceptor was not recognized by monoclonal antibodies directed against conformation-dependent epitopes. These findings suggest that the Gly31 to Arg31 mutant is involved in the insulin receptor dysfunction seen in the Leprechaun patient. The mutation seems to alter the conformation of the receptor in such way that the transport of the proreceptor to the Golgi compartment, where proteolytical processing occurs, is inhibited.

The insulin receptor is a heterotetrameric protein, composed of two α and two β chains. The α chain is located extracellularly and is involved in ligand binding. The β chain is a transmembrane protein, with a cytoplasmic domain that exhibits tyrosine kinase activity (1-4). The subunits are derived from one single precursor consisting of sequences for the signal peptide, the α chain and the β chain. In the endoplasmic reticulum, the signal peptide is removed and glycosylation occurs to form a 190-kDa core glycosylated precursor. In the Golgi apparatus several subsequent glycosylation steps take place to yield a 210-kDa proreceptor. This proreceptor is subsequently cleaved to pre-α and pre-β chains, which are further glycosylated to yield mature α and β chains.

The chains remain interlinked by disulfide bonds and are transported to the cell surface (5, 6). Binding of insulin to the α chain induces a conformational change that results in activation of the tyrosine kinase.

A wide range of patients with clinically different forms of insulin resistance, due to mutations in the insulin receptor, have been described over the past years (7-16). Of those, patients with Leprechaunism exhibit the most severe form of insulin resistance. We previously described such a patient, Leprechaun Geldermalsen (12, 17), having a homozygous proline for leucine substitution at position 233 (Pro233). The mutation causes impaired receptor processing and transport of the proreceptor to the cell surface.

Another Leprechaun patient described by us, patient Helmond, shows markedly reduced insulin binding to intact fibroblasts but significant receptor autophosphorylation when the glycoprotein fraction is used (18). The insulin stimulated uptake of 2-deoxyglucose in fibroblasts is impaired but a normal response is seen when insulin-like growth factor-1 is used (19). These data suggest a defect associated with the insulin receptor.

In order to analyze the defect in the insulin receptor, we have directly sequenced the exons encoding the insulin receptor after amplification by the polymerase chain reaction. One heterozygous nucleotide substitution was found in exon-2, changing a glycine into an arginine at position 31 (Arg31). This mutation was also present in the maternal line in individuals with decreased insulin binding to cultured fibroblasts and in vivo hyperinsulinemia after an oral glucose tolerance test. In the paternal line, all subjects had an insulin binding defect within the normal range and the mutation was absent.

To further investigate the effect of the Arg31 substitution on insulin receptor properties, we have created Chinese hamster ovary (CHO) cell lines overexpressing the mutant Arg31 insulin receptor and the properties of the mutant receptor were studied.

EXPERIMENTAL PROCEDURES

Materials—Taq polymerase and thermocycler were from Perkin-Elmer/Cetus. Deoxynucleotides, lactoperoxidase, and glucose oxidase were from Boehringer Mannheim. Other enzymes were from Pharmacia LKB Biotechnology Inc. [35S]Methionine, 32P-labeled nucleotides, 125I Na, and A14-mono[3H]Tiodo insulin from Amersham. Bovine serum albumin, radioimmunoassay grade, and Sequenase version 2.0 were from United States Biochemical, Disuccinimidyl suberate (DSS)

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1 The abbreviations used are: CHO, Chinese hamster ovary; DSS, disuccinimidyl suberate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
was from Pierce Chemical Co. Glycoprotein was prepared as described previously. Monoclonal human insulin was obtained from Nove, Denmark. Immune precipitates were analyzed on 8% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) using as markers myosine (220 kDa), β-galactosidase (120 kDa), phosphorylase (97 kDa), bovine serum albumin (68 kDa), and ovalbumin (45 kDa). The protein markers were obtained by using a high molecular weight kit from Bio-rad. Dried SDS-PAGE gels were exposed to Kodak-Xar films using intensifying screens. In case of 32P-labeling, the gels were impregnated with Amplify (Amer sham).

Subject—Patient Helmond, a Caucasian female with Leporechiasm who died at the age of four months, was described in detail previously (18).

Polymerase Chain Reaction and Sequencing—Genomic DNA was isolated from fibroblasts of patient Helmond and her relatives using standard techniques. 1 µg of genomic DNA was subjected to 30 cycles of polymerase chain reaction amplification using the intron primer sets according to Seino et al. (20). Each reaction was performed in a volume of 100 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 10 µg/ml gelatin; 50 µM each of dATP, dGTP, dCTP, and dTTP; 0.15 µg each of oligonucleotide primer and 2.5 units of Taq DNA polymerase. After initial denaturation at 94 °C for 5 min, the samples were subjected to 30 cycles of amplification with an automated DNA thermal cycler; annealing at 50-60 °C for 2 min, extension at 72 °C for 2 min, and denaturation at 94 °C for 1 min. After purifying the individual exons by electrophoresis on Nu sieve low melting agarose (FMC Bioproducts), 10 ng of DNA of each exon was directly sequenced according to Casanova et al. (21) using the Universal Biological Sequencing kit Version 2.0.

Oligonucleotide Hybridization—We used two different oligonucleotides, one complementary to the wild type antisense strand (5'- GTGTGATCGAAGAATTGG-3') and one to the mutant anti-sense strand (5'-GTGTGATCGAGAAATTCG-3'). The oligonucleotides were labeled with 32P by T4 polynucleotide kinase according to Maniatis et al. (22). 0.1 µg of amplified exon-2 DNA from the patient, her family members and controls were denatured for 5 min at 95 °C and spotted on Bio-trace filters. Filters were dried and hybridized to filters containing 0.25 pg of exon-2 DNA: control (C), the proband (III-1), the father (II-1), the mother (II-2), and grandparents (I-1-1-4). A, hybridization with the mutant probe or the wild type probe (B). Numbers refer to individuals in the pedigree shown in Fig. 3.

Fig. 1. Partial nucleotide sequence of insulin receptor exon-2. Genomic DNA from patient Helmond was amplified and directly sequenced. A heterozygous change in codon 31 is seen (GGA→AGA).

RESULTS

Sequence Analysis—To detect mutations in the insulin receptor of patient Helmond DNA-sequence analysis was applied to direct sequencing amplified exons encoding the insulin receptor. One heterozygous nucleotide substitution changing codon GGA into AGA was found in exon-2 resulting in substitution of arginine for glycine at position 31 (Fig. 1).

Inheritance Pattern—By allele-specific oligonucleotide hybridization the pattern of inheritance of the Arg2 mutation in the pedigree was examined. As shown in Fig. 2 only the

FIG. 2. Allele-specific hybridization to amplified exon-2 DNA. Two oligonucleotides encoding the wild type (AGA) and the mutant codon (AGA) were end-labeled with 32P]ATP and hybridized to filters containing 0.25 µg of exon-2 DNA: control (C), the proband (III-1), the father (II-1), the mother (II-2), and grandparents (I-1-1-4). A, hybridization with the mutant probe or the wild type probe (B). Numbers refer to individuals in the pedigree shown in Fig. 3.
proband, the mother and the maternal grandfather are heterozygous for the Arg25-mutation. This mutation co-segregates with a decreased binding of insulin to fibroblasts (Fig. 3). All other family members are homozygous for the wild type Gly25 sequence.

The father of Leprechaun Helmond is a type I diabetic individual with normal insulin binding. The paternal grandmother has type II diabetes mellitus with slightly decreased insulin binding that falls, however, within the normal range.

Transfection Experiments—CHO cells were stably transfected by an SV40 driven expression vector which carries cDNA encoding either the Gly25 wild type (WT-HIR CHO) or the Arg25 mutant (Arg25-HIR CHO) insulin receptor. Clonal lines expressing the insulin receptor were selected by metabolic labeling and immune precipitation with a monoclonal antibody (RPN 538); lane 2, polyclonal; lane 3, nonimmune serum.

The polyclonal precipitates the α and β chains together with a 210-kDa protein in the WT-HIR CHO cell lines. In the Arg25-HIR CHO cell lines only a 210-kDa protein is specifically precipitated by the antibody. No α and β chains were detectable (Fig. 4A). This immune precipitation pattern was seen in several cell lines. As shown previously, the 210-kDa protein is the proteolytically unprocessed precursor of the insulin receptor (17). Thus the Arg25-HIR CHO cell lines only express the insulin proreceptor. Furthermore, two different monoclonal antibodies against the insulin receptor which recognize conformational epitopes on the α chain were unable to precipitate the mutant proreceptor (Fig. 4B). This indicates that the mutation affects the conformation of the receptor α chain.

We also tested whether the mutant 210-kDa proreceptor was retained by wheat germ lectin-Sepharose columns. For that, 35S-labeled cell lysates from CHO cells expressing the mutant receptor were passed over wheat germ agglutinin-Sepharose. The flow through and the N-acetylglucosamine eluate was subjected to immune precipitation with polyclIR. Most of the mutant 210-kDa proreceptor appeared in the glycoprotein fraction (not shown).

Kinetics of Proreceptor Biosynthesis—The kinetics of the biosynthesis of the wild type and mutant insulin receptor in transfected CHO cells were studied by pulse-chase labeling experiments. The transfected cells were labeled for 20 min with [35S]methionine and incubated for varying times with unlabeled methionine. Cells were then solubilized, immune precipitated with polyclIR, and analyzed on an SDS-PAGE gel (Fig. 5).

At the start point of the chase few proreceptors in the WT-HIR CHO cells, as indicated by the 210-kDa band, are seen, whereas a much more pronounced band appears at 210 kDa in the Arg25-HIR CHO cells. During the chase the WT-HIR proreceptor was largely converted into α and β chains. The Arg25-HIR proreceptor remained at the same position with little decline in intensity.

These data indicate that there is increased stability of the proreceptor in the Arg25-mutant-transfected CHO cells and no processing to the individual α and β chains. From the decay in intensity, we estimate the half-life of the mutant proreceptor to be at least 8 h.

Insulin Binding—Because the insulin receptor consists of a tetramer composed of two αβ-dimers, the Arg25 mutant receptor may interfere with the functional expression of the endogenous CHO insulin receptors by forming nonfunctional hybrids. After confirming overexpression of the mutant receptor in CHO cells by metabolic labeling and immune precipitation, we tested insulin binding to parental CHO, WT-HIR CHO, and Arg25-HIR CHO cells (Table 1).

WT-HIR CHO cells showed an approximately 40-fold increase in binding compared to parental CHO cells, Arg25-HIR CHO cells showed characteristics comparable to the parental CHO cells. Therefore, overexpression of the Arg25 mutant does not interfere with the expression of functional endogenous insulin receptors on the cell surface. In addition, these

![Fig. 3. The pedigree of patient Helmond showing, in addition, insulin binding to fibroblasts as a percentage of insulin binding to control fibroblasts. BM, diabetes mellitus.](image)

![Fig. 4. Biosynthesis of insulin receptors in WT-HIR and Arg25-HIR CHO cells. Cells were labeled for 8 h with [35S]methionine and proteins were immune precipitated from each cell lysate. A, immune precipitation by polyclIR. Lane 1, the WT-HIR CHO cells; lane 2, the Arg25-HIR CHO cells. B, immune precipitation of the insulin receptor from Arg25-HIR CHO cells. Lane 1, monoclonal antibody (RPN 538); lane 2, polyclonal; lane 3, nonimmune serum.](image)

![Fig. 5. Pulse-chase labeling of WT-HIR CHO and Arg25-HIR CHO cells. WT-HIR CHO (WT) and Arg25-HIR CHO (MT) cells were labeled for 20 min with [35S]methionine followed by incubation with nonradioactive methionine for 0, 1, 3, and 7 h. Cell lysate was immune-precipitated with polyclIR and analyzed by SDS-PAGE and fluorography. The positions of the proreceptor (210 kDa), the α chain (135 kDa), and the β chain (95 kDa) are indicated. A control nonimmune precipitation experiment is shown in Fig. 4B.](image)
TABLE I
Specific binding of 125I-insulin to parental CHO, WT-HIR CHO, and Arg13-HIR CHO cells

Values are averages of triplicate experiments. Nonspecific binding, i.e. radioactivity bound in the presence of 1 μM nonradioactive insulin is subtracted. Standard deviations are indicated.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Insulin bound 10^-15 mol/10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-parental</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>CHO-WT-HIR</td>
<td>16 ± 0.60</td>
</tr>
<tr>
<td>CHO-Arg13-HIR</td>
<td>0.35 ± 0.03</td>
</tr>
</tbody>
</table>

![Figure 6](image)

**Fig. 6.** 125I cell surface iodination of WT-HIR CHO and Arg13-HIR CHO cells. After lactoperoxidase-catalyzed cell surface iodination, the cell lysate was immune-precipitated with either polyclR (lanes 1 and 3) or nonimmune serum (lanes 2 and 4). Lanes 1 and 2, Arg13-HIR CHO cells; lanes 3 and 4, WT-HIR CHO cells. Positions of the α chain (135 kDa), the β chain (95 kDa), and the proreceptor (210 kDa) are indicated.

![Figure 7](image)

**Fig. 7.** Affinity cross-linking of 125I-insulin to its receptor. 10 μg of lectin-purified glycoprotein from parental CHO, WT-HIR CHO, and Arg13-HIR CHO cells was incubated with 50 pm 125I-insulin in the absence (lanes 2, 4, and 6) or presence (lanes 1, 3, and 5) of 1 μM nonradioactive insulin. Cross-linking was by 0.2 mM DSS. After immune precipitation the samples were analyzed by SDS-PAGE and autoradiography. Lanes 1 and 2, parental CHO cells; lanes 3 and 4, WT-HIR CHO cells; lanes 5 and 6, Arg13-HIR CHO cells. Position of the α chain (135 kDa) and the proreceptor (210 kDa) are indicated. Exposure was long enough to detect endogenous insulin receptor α chains from CHO cells.

results indicate that the mutant receptor does not generate additional insulin binding sites on the cell surface.

Cell Surface Iodination—The improperly processed proreceptor can either be transported to the cell surface or arrested in an intracellular compartment. We studied these possibilities by lactoperoxidase-catalyzed cell surface iodination with 125I-Na. The appearance of radiolabeled insulin receptor on the cell surface was measured by immune precipitation, SDS-PAGE, and autoradiography (Fig. 6).

In the WT-HIR CHO cells the α chain became strongly labeled while in the Arg13-HIR CHO cells no labeling of either the α chain or the 210-kDa precursor was detected. So it seems that the Arg13 proreceptor is arrested in an intracellular compartment.

Cross-linking—The possibility exists that the proreceptor, though not appearing on the cell surface, still has a functional insulin binding site. To examine this possibility, 125I-insulin was cross-linked to lectin-purified glycoprotein, prepared from parental, WT-HIR, and Arg13-HIR CHO cells. Similar concentrations of glycoprotein were used. Cross-linking was induced by DSS (Fig. 7).

In the WT-HIR CHO cells the 135-kDa band is specifically labeled, indicating binding of insulin to the α chain. In the Arg13-HIR CHO cells α chain labeling is comparable to the parental cells. There was no specific labeling of the α chain or shift of labeling to the 210-kDa band. Therefore, the Arg13 mutant proreceptor seems not capable of binding insulin.

**Autophosphorylation**—The Arg13 mutant proreceptor was also studied for its ability to undergo autophosphorylation, both with or without 50 nM insulin (Fig. 8). Glycoprotein fractions were used.

In case of WT-HIR CHO cells a strong signal at 95 kDa, i.e. the β chain, is observed in presence of insulin. The Arg13-HIR CHO cells had an autophosphorylation pattern similar to the parental CHO cells. Also no signal was detected at the position of the precursor. This implies that Arg13 proreceptor does not undergo autophosphorylation, both in the absence or presence of insulin.

**DISCUSSION**

We previously reported on functional aspects of the insulin receptor in fibroblasts from Leprechaun Helmond and her family members (18). Those studies suggested the presence of an inherited mutation in the insulin receptor in the patient and the maternal line. We found a distinct reduced insulin binding to the fibroblasts of the patient (18% of control). In this study we show that her mother and maternal grandfather also have decreased insulin binding values of 32 and 28%, respectively. In addition, we have identified a mutation in the insulin receptor of Leprechaun Helmond changing a glycine into an arginine at position 31. By oligonucleotide hybridization of amplified genomic DNA we detected the mutation in the patient and members of the maternal line with reduced insulin binding. These individuals were heterozygous for the Arg13 mutation. Except for the proband who shows severe insulin resistance these individuals show no manifest clinical symptoms though oral glucose tolerance tests show hyperinsulinemia with normoglycemia. This situation is similar to the situation in relatives of Leprechaun Geldermalsen where it was shown that individuals with only one allele for a functional insulin receptor had hyperinsulinemia and normoglycemia (24). It is expected that the proband has an additional defect inherited from the father causing the disease. Despite unambiguous sequencing of the complete coding sequence of the insulin receptor and splice sides, no additional mutation was detected. By converting mRNA of the patient into cDNA, followed by amplification of insulin receptor cDNA and hybridization with oligonucleotides specific for the wild type and mutant receptor sequence we found that both the wild type and mutant allele are expressed. At this moment we have no experimental data to explain why the proband

![Figure 8](image)

**Fig. 8.** Insulin-stimulated autophosphorylation of insulin receptors in parental CHO, and Arg13-HIR CHO cells. 4 μg of glycoprotein was incubated with (lanes 1, 3, and 5) or without (lanes 2, 4, and 6) 50 nM insulin. Autophosphorylation was initiated by adding [γ-32P]ATP. The insulin receptor was immune precipitated with polyclR and analyzed by SDS-PAGE and autoradiography. Lanes 1 and 2, WT-HIR CHO cells; lanes 3 and 4, Arg13-HIR CHO cells; lanes 5 and 6, parental CHO cells. Position of β chain (95 kDa) and proreceptor (210 kDa) are indicated.
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has the leprechaun phenotype. A surprising observation, which we made previously (18), is the presence of insulin receptors in the glycoprotein fraction of fibroblasts from this patient, despite low insulin binding to these cells. These insulin receptors show a normal dose-response curve for insulin-stimulated autophosphorylation. This observation agrees with a situation where the paternally encoded insulin receptor is subject to enhanced internalization. A situation with a certain resemblance is seen in leprechaun Ark 1. There, one allele has a nonsense codon, whereas the other allele encodes an insulin receptor with a Glu for Lys substitution. In transfection experiments this mutant receptor was found to mediate signalling but was very susceptible for insulin-stimulated degradation (25). In Leprechaun Helmond we did not detect a mutation in the paternally encoded receptor allele which could explain enhanced internalization of the receptor. However, this patient may have an additional mutation outside the receptor gene which could disturb the process of receptor internalization or intracellular routing in such a way that recycling of internalized receptors to the plasma membrane is impaired. An additional possibility has recently emerged from the observation that insulin receptors with exon-11 (the B-isotype) recycle more slowly to the cell surface than the A-isotype (minus exon-11) (26). Splicing of the primary receptor transcript may be such in our patient that predominantly B-receptors are formed. Experiments are aimed to investigate these possibilities.

The Arg mutation is associated with reduced insulin binding. So a causal linkage between the Arg mutation and the binding defect seemed likely. By overexpressing the Arg mutant in CHO cells we created cell lines to study the functional properties of the Arg receptor. Insulin binding to the Arg-HIR-transfected cells was similar as binding to parental cells showing that receptors with this mutation do not contribute to insulin binding. It is remarkable that fibroblasts of the mother (II-2) and grandfather (I-3) have insulin binding values of nearly 25% instead of the expected 50%. An explanation could be the formation of hybrids between WT and mutant receptors, which are not transported, leaving only 25% WT-αβ receptors for binding. This possibility could explain the insulin binding values but does not agree with the results of the transfection experiment in CHO cells. The expression of the Arg receptor is approximately 40 times higher than expression of endogenous hamster receptors. If hybrids can form with the Arg receptor, it is expected that all the endogenous receptors will be present in hybrid form, resulting in a loss of insulin binding to the CHO cells. This is not observed. Insulin binding to individual fibroblasts show some variations and these naturally occurring fluctuations may cause the lower than 50% binding to fibroblasts of I-3 and II-2.

The pulse-chase experiments show a faster appearance of the mutant 210-kDa precursor than the WT precursor. It seems less likely that the actual rate of biosynthesis of the mutant precursor is higher. The rate of appearance of the proreceptor in a pulse-chase experiment is determined by the rate of biosynthesis and the conversion rate into pro-α and pre-β subunits. As the mutant proreceptor is converted only very slowly, it is expected that it will show up faster in the pulse-chase experiment.

The folding of the insulin receptor has been predicted on theoretical grounds (27). Gly residue located in a hinge region of the insulin receptor protein. Substituting arginine for glycine in this region is expected to have a profound impact on the folding of the insulin receptor α chain. This assumption is substantiated by the absence of recognition by two monoclonal antibodies recognizing conformation dependent epitopes on the α chain. Since no cross-linking of 125I-insulin to the proreceptor was observed we assume that the mutation also interferes with proper folding of the insulin binding site. The absence of insulin binding prevents insulin-stimulated autophosphorylation.

The lack of proteolytic cleavage of the Arg mutation, which is located far upstream to the cleavage side, may also be explained by the changed conformation. It has been reported that the transport of proteins through the Golgi compartments can be affected by changes in the protein folding. In case of the insulin receptor an impaired transport prevents the proreceptor from migrating to the Golgi compartment where proteolytic cleavage take place. That normal transport to the cell surface does not require proteolytic cleavage is shown by several studies. Yoshimasa et al. (7) found that a naturally occurring mutation in the tetraspanic cleavage side of the αβ-proreceptor decreases only proteolytic cleavage of the proreceptor but not the transport to the cell surface. Williams et al. (28) constructed insulin receptor mutants lacking the tetraspanic cleavage site. A large fraction of this mutant receptor appeared as a 205-kDa αβ-proreceptor on the cell surface. Several different mutations in the insulin receptor are described that are, like the Arg mutant, associated with incomplete proteolytic processing and defective transport. These mutations are: Pro (17), Lys (29), and Arg (30). The Pro for Leu substitution at position 233 occurs within the cysteine-rich cluster of the receptor. The Lys for Asn substitution at position 15 has been suggested to interfere with the formation of an α-helical structure in that region of the receptor (29). This α-helix is flanked by 2 cysteine residues at positions 8 and 26. Position 209, where the Arg for His mutation is located far upstream to the cleavage side, may also be explained by the changed conformation. It has been reported that the transport of proteins through the Golgi compartment can be affected by changes in the protein folding. In case of the insulin receptor an impaired transport prevents the proreceptor from migrating to the Golgi compartment where proteolytic cleavage take place. That normal transport to the cell surface does not require proteolytic cleavage is shown by several studies. Yoshimasa et al. (7) found that a naturally occurring mutation in the tetraspanic cleavage side of the αβ-proreceptor decreases only proteolytic cleavage of the proreceptor but not the transport to the cell surface. Williams et al. (28) constructed insulin receptor mutants lacking the tetraspanic cleavage site. A large fraction of this mutant receptor appeared as a 205-kDa αβ-proreceptor on the cell surface. Several different mutations in the insulin receptor are described that are, like the Arg mutant, associated with incomplete proteolytic processing and defective transport. These mutations are: Pro (17), Lys (29), and Arg (30). The Pro for Leu substitution at position 233 occurs within the cysteine-rich cluster of the receptor. The Lys for Asn substitution at position 15 has been suggested to interfere with the formation of an α-helical structure in that region of the receptor (29). This α-helix is flanked by 2 cysteine residues at positions 8 and 26. Position 209, where the Arg for His substitution occurs, is preceded by 2 cysteine residues. It is plausible that these mutations all interfere with the formation of correct disulfide bonds and thereby inhibit the transport of the proreceptor to the Golgi compartment where the proteolytic cleavage occurs.

The increasing number of transport-defective mutant insulin receptors indicates that in a significant fraction of patients with insulin resistance, resulting from decreased insulin binding, the mutations may act by preventing the appearance of the insulin receptor on the cell surface rather than by directly affecting the insulin binding site.

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REFERENCES

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