Sequencing analysis of insulin receptor defects and detection of two novel mutations in \textit{INSR} gene☆

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Mutations in the insulin receptor gene cause the inherited insulin resistant syndromes Leprechaunism and Rabson–Mendenhall syndrome. These recessive conditions are characterized by intrauterine and post-natal growth restrictions, dysmorphic features, altered glucose homeostasis, and early demise. The insulin receptor gene (\textit{INSR}) maps to the short arm of chromosome 19 and is composed of 22 exons. Here we optimize the conditions for sequencing this gene and report novel mutations in patients with severe insulin resistance.

\textbf{Methods:} PCR amplification of the 22 coding exons of the \textit{INSR} gene was performed using M13-tailed primers. Bidirectional DNA sequencing was performed with BigDye Terminator chemistry and M13 primers and the product was analyzed on the ABI 3100 genetic analyzer. Data analysis was performed using Mutation Surveyor software comparing the sequence to a reference \textit{INSR} sequence (Genbank NC_000019).

\textbf{Results:} We sequenced four patients with Leprechaunism or Rabson–Mendenhall syndromes as well as seven samples from normal individuals and confirmed previously identified mutations in the affected patients. Three of the four mutations identified in this group caused premature insertion of a stop codon. In addition, the \textit{INSR} gene was sequenced in 14 clinical samples from patients with suspected insulin resistance and one novel mutation was found in an infant with a suspected diagnosis of Leprechaunism.

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Discussion: Leprechaunism and Rabson–Mendenhall syndrome are very rare and difficult to diagnose. Diagnosis is currently based mostly on clinical criteria. Clinical availability of DNA sequencing can provide an objective way of confirming or excluding the diagnosis.

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1. Introduction

The insulin receptor is a membrane protein composed of two extracellular α subunits that bind insulin and two β subunits which span the plasma membrane and have an intracellular tyrosine kinase domain [1,2]. Insulin binding to the α-subunits causes a conformational change that results in the activation of the kinase activity of the β-subunits with subsequent autophosphorylation and activation of kinase activity toward intracellular substrates [1,2]. A single gene codes for both subunits. The resulting preprotein is post-translationally cleaved into mature alpha and beta subunits that assemble together as a heterotetramer to generate the mature insulin receptor [1–3]. The INSR gene maps to the short arm of chromosome 19 and is composed of 22 exons. Alternative splicing of the 36 base pair exon 11 results in two isoforms which differ in sequence at the C-terminal end of the insulin-binding alpha-subunit [3].

Mutations in INSR cause the insulin-resistant syndromes Leprechaunism, also known as Donohue syndrome [4], Rabson–Mendenhall syndrome and type A insulin resistance [5,6]. Leprechaunism, (OMIM 246200), the most severe of the insulin resistant syndromes, is characterized by intrauterine growth restriction (IUGR), loss of glucose homeostasis, hyperinsulinemia, and dysmorphic features, with prominent eyes, thick lips, upturned nostrils, low-set posteriorly rotated ears, thick skin with lack of subcutaneous fat, distended abdomen, and enlarged genitalia in the male and cystic ovaries in the female [7–9]. Cells from most patients with Leprechaunism have markedly reduced insulin binding, although exceptions were reported [10,11].

The slightly less severe Rabson–Mendenhall syndrome (OMIM 262190) was first described in three siblings with dental and skin abnormalities, abdominal distension, early dentition, coarse senile-looking facies, striking hirsutism, intellectual disability, prognathism, thick fingernails and acanthosis nigricans. Insulin-resistant diabetes mellitus, ketoacidosis, intercurrent infections, pineal hyperplasia and ovarian tumor [12]. Children have initial postprandial hyperglycemia and fasting hypoglycemia, caused by inappropriately elevated insulin levels at the time of fasting [6,13]. Patients with Rabson–Mendenhall syndrome can survive beyond 1 year of age and, with time, develop constant hyperglycemia followed by diabetic ketoacidosis and death. This is accompanied by a progressive decline of insulin levels, which become insufficient to prevent liver glucose synthesis and release of fatty acids by adipocytes [13].

Mutations in the insulin receptor can cause disease with a dominant pattern of inheritance as well. For example, a mutation (p.Gly996Val) in a conserved Gly-X-Gly-X-X-Gly motif impairs tyrosine kinase activity of the insulin receptor and is associated with insulin-resistant diabetes mellitus and acanthosis nigricans, suggesting a dominant-negative pathogenesis [14–16]. A different mutation (p.Arg1174Gln) with unknown functional effects in INSR is implicated in familial hyperinsulinemic hypoglycemia type 5 in a few patients (HHF5) [17].

Leprechaunism and Rabson–Mendenhall syndrome are inherited as autosomal recessive traits. There is some correlation between genotype and phenotype, with mutations that markedly impair insulin binding resulting in the most severe phenotypes, while the presence of at least one mutation leaving residual insulin binding activity is associated with longer survival [6,18]. Definitive genotype–phenotype correlation for INSR defects is difficult to establish primarily due to the rarity of these syndromes [6], a paucity of functional studies to determine the effect of mutations on insulin binding or signaling, and difficulty in establishing a precise molecular diagnosis due to the lack of clinically validated INSR gene sequencing [6,19].

Herein we develop a clinically validated sequencing method to discover mutations in the INSR gene. Bidirectional sequencing with BigDye terminator and M13 primers was used to examine mutations in the coding regions and exon–intron boundaries of the INSR gene. A combination of the biochemical and DNA tests can provide accurate diagnosis for the insulin receptor deficiency.
2. Materials and methods

2.1. Patients/samples

DNA from 11 unrelated individuals (7 controls and 4 patients with Leprechaunism) was used to determine performance characteristics of this INSR full gene sequencing assay. Of these four patients with Leprechaunism, three of them, referred to here as 452, NY1, and 5880, had previously been described [6,7,23]. Fibroblasts from each of these patients were received and DNA was extracted by MagNA Pure. The fourth patient with Leprechaunism, SLC, was not previously described but fit the clinical criteria. The diagnosis of Leprechaunism for all four patients was established from clinical presentation (failure to thrive, growth retardation, markedly elevated insulin levels, hirsutism, and acanthosis nigricans) and markedly reduced insulin binding to patients’ fibroblasts. The samples were de-identified following an

| Primer name | Sequence |
|-------------|----------|
| IR E1F      | tgaacaagcaggccagtGGCTCTGATCGAGGAGA  |
| IR E1R      | cgaagggcgtgtaACATCACATACAGA  |
| IR E2F #2   | tgaacaagcaggccagtTCTCCTGGTCTAATAG  |
| IR E2R #2   | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E3F #2   | tgaacaagcaggccagtTCTCCTGGTCTAATAG  |
| IR E3F Int  | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E3R      | cgaagggcgtgtaACATCACATACAGA  |
| IR E4F      | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E4R      | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E5F      | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E5R      | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E6F #2   | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E6R      | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E7F #2   | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E7R      | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E8F      | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E8R #2   | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E9F      | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E9R      | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E10F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E10R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E11F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E11R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E12F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E12R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E13F #2  | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E13R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E14F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E14R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E15F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E15R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E16F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E16R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E17F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E17R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E18–19F  | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E18–19R  | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E20F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E20R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E21F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E21R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
| IR E22F     | tgaacaagcaggccagtGCTCGAGATGCTGAGAAC  |
| IR E22R     | cgaagggcgtgtaCACAGCTGGAACATCATAG  |
Institutional Review Board (IRB)-approved protocol. Fourteen additional samples referred to the ARUP Sequencing Laboratory by the patients' clinicians for INSR mutation detection were sequenced and analyzed.

2.2. DNA sequencing of the INSR gene

DNA was extracted from leukocytes in blood using MagNAPure Compact instrument (Roche Applied Science, Indianapolis, IN). Nucleic acid sequencing for the INSR gene coding region was performed by standard dideoxy termination. PCR primers were developed for the 22 exons of the INSR isoform containing exon 11 (NM_002082). Eighteen sets of PCR primers used in this validation were previously published [20], however, in the current study four sets of primers were re-designed to optimize PCR and sequencing results (see Table 1). We added another internal primer set to exon three interior to the homopolymer region to obtain cleaner sequence. In addition, exons 18 and 19 were consolidated into one amplicon, polymerase chain reaction of the 22 coding exons of the INSR gene was performed using M13-tailed primers Premix D (Epigence, Madison, WI), and Platinum Taq (Invitrogen, Carlsbad, CA) using PCR conditions shown in Table 2 below. Unused PCR primers and unincorporated nucleotides were inactivated by incubation with ExoSAP (USB Corporation, Cleveland, OH). Bidirectional DNA sequencing was performed with BigDye Terminator chemistry (ABI, Foster City, CA) and M13 primers (IDT, Coralville, IA) and the product was analyzed on the ABI 3730. Data analysis was performed using Mutation Surveyor software (SoftGenetics, State College, PA) and GenBank reference sequence NG_008852.1.

3. Results

3.1. INSR mutation update

The INSR gene product contains 120 kilobases and is composed of 22 exons. There are three transcription initiation sites located at 276, 282 and 283 base pairs upstream of the translation initiation site. The alpha subunit is encoded by exons one through 11 (and part of exon 12) whereas the beta subunit is encoded by exons 12–22 [1,2]. The insulin receptor is synthesized as a single protein that is post-translationally cleaved at a four amino acid site (p.759_762, RKRR, encoded by exon 12) to generate the mature alpha and beta subunit.

The INSR gene product contains a leader sequence of 27 amino acids. Cleavage of these amino acids results in the mature active protein. As a result of this cleavage, the nomenclature of reported variations differs depending on the author, time of publication, and source of their reference DNA sequence. For this reason, we reviewed the published literature for all known INSR variations to determine the consistent amino acid position using the current nomenclature, in both the immature and the mature protein. Absolute nucleotide positions were kept consistent with the beginning of the cDNA regardless of protein cleavage (recommendations of the Human Genome Variation Society, http://www.hgvs.org/rec.html). In addition, the INSR gene has two isoforms that differ only by the 12 amino acids encoded by the alternatively spliced exon 11. These isoforms have slightly different reported biological activity and different abundance in different tissues with the isoform containing exon 11 being predominant in the liver; the other in leukocytes; with similar expression levels in most other tissues such as skeletal muscle, placenta and adipose tissue [21].
To date, there are 132 reports of disease causing mutations in the \textit{INSR} gene in the literature (Table 4). The majority of the mutations (64%, or 85 of 132) are missense mutations, 13% (17 of 132) are nonsense mutation, 4.7% are splice site mutations, 8.3% are deletions (11/132), 2.3% are insertions (3/132), 1.5% are insertions and deletions (indel, 2/132), 5.3% are gross deletions or complex gene rearrangements (7/132) (Fig. 1). Most of the mutations located in the first 11 exons result in Leprechaunism while the mutations in the beta subunit are found more frequently in patients with Rabson–Mendenhall syndrome.

3.2. Sequencing INSR

Four patients with known mutations were verified by the above sequencing protocol. The first patient, NY1, with clinically-confirmed Leprechaunism \cite{6,22}, had a homozygous G to T variation at nucleotide 451 converting Glu 151 to a premature stop codon (c.451G\textrightarrow{}T, p.Glu151term). The second patient, 452, was a female infant with symptoms including repeated transient hypoglycemic episodes, prominent female genitalia, marked hirsutism, breast hyperplasia, loose and pachydermatous skin, decreased adipose tissue, acanthosis nigricans, and abdominal distention \cite{7}. Sequencing results showed a heterozygous C to T nucleotide change at position 1195 coding for a premature stop codon at amino acid position 399 (c.1195C\textrightarrow{}T, p.Arg399term). A second mutation could not be detected by the assay as in the initial publication \cite{7}. The third patient, 5880, had physical features of Leprechaunism and his lymphoblasts had a 90% decrease in the number of insulin receptors. This patient had a heterozygous C to T nucleotide change at position 2734 resulting in a change of arginine 924 to a premature stop codon (c.2770C\textrightarrow{}T, p.Arg924term) \cite{23}. A second mutation could not be found even in this patient as in the original manuscript \cite{24}.

The forth patient, SLC, died before one year of age and had physical features of Leprechaunism. Insulin binding was reduced to about 4% of normal in fibroblasts from this patient. A novel G to T missense mutation was identified at nucleotide position 425 resulting in a change of glycine 142 to a valine (c.425G\textrightarrow{}T, p.Gly142Val). Computational prediction with the program Polyphen 2 (Harvard) predicts that a glycine to valine amino acid change at this position is “possibly damaging” with a score of 0.814 while SIFT (J Craig Venter Institute) predicts that the substitution is “damaging”. A second mutation could not be identified in this patient either.

Seven additional samples from normal, healthy individuals displayed no \textit{INSR} variants. However all samples (as well as the clinical samples above) were found to have a benign polymorphism at nucleotide position 5 changing alanine 2 to glycine (c.5C\textrightarrow{}G, p.Ala2Gly).

An additional fourteen clinical samples (one sample from cultured amniocytes, four samples from pediatric patients and nine from adult patients) were referred to our lab for \textit{INSR} sequencing. The clinical phenotype and laboratory results are summarized in Table 3. According to patient history received by ARUP with the amniotic sample, it was previously tested for deletions and duplications using a SNP array at another laboratory and was found to have a 63 kb deletion at 19p13.2 (7,143,507–7,206,857), including deletion of several exons of the \textit{INSR} gene. Sequencing analysis detected no additional mutations. The 13

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Summary of types of mutations found in the \textit{INSR} gene.}
\end{figure}
pediatric and adult patients presented with anomalies including, intra-uterine growth restriction (IUGR), failure to thrive (FTT), dysmorphic features, distended abdomen, and acanthosis nigricans. Although the major symptom was insulin resistance, the nine oldest patients tested were disproportionately female (7:2) with gynecological symptoms including menstrual irregularities and cystic ovaries. One 16 year old male patient had a history of IUGR, FTT, dysmorphic features, and poor response to exogenous insulin. Thirteen samples had no mutation detected by Sanger sequencing in the coding regions and exon/intron boundaries. An eleven week old boy with suspected Leprechaunism was homozygous for a variant of unknown clinical significance, c.2971C>T, p.Leu991Ile. For this patient, no positions of heterozygosity were observed in INSR, therefore we cannot rule out a partial or complete gene deletion. The patient was in intensive care and presented with IUGR, bilateral club feet, congenital hydrocephalus and dysmorphic features. Patient had only the right kidney and renal tubular acidosis. The patient had sporadic hypoglycemia and was noted to have glucose levels decreasing to 40 mg/dL range after 4–5 h of fasting, but given the age and size of the patient, this is of uncertain clinical significance. This patient also had elevated beta-hydroxybutyric acid of 14.1 mg/dL (reference range: 0.0–3.0) and a random insulin level of 1 μU/mL (reference range: 3–19 μU/mL). This variant (rs150114699) has been seen in the general population with a frequency of 0.4% in 1000 genomes and 0.6% in 6500 exomes in African Americans. The homozygous variant, c.2971C>T, p.Leu991Ile, has never been reported in the literature; sequence prediction programs give conflicting results about whether this substitution is likely to be deleterious (SIFT: deleterious; PolyPhen2: benign at score: 0.442). The next residue, Y992 is a conserved phosphorylation site in a highly conserved region (DGPLGPLyASSNPEY, http://www.phosphosite.org/siteAction.do?id=13426). The putative amino acid change

Table 3
Clinical information and laboratory results for patient samples sequenced in this study.

| Patient | Age   | Ethnicity     | Gender | Clinical and other findings                                                                 |
|---------|-------|---------------|--------|-----------------------------------------------------------------------------------------------|
| 1       | Fetus | Asian         | NA     | Reported advanced maternal age. A SNP array detected a 63 kb deletion involving deletion of exons 3–11 of the INSR gene: 19p13.2(7,143,507–7,206,857)x1; GRCh37/hg 19 sequencing of the coding exons ruled out second mutation |
| 2       | 11 weeks | African-American | M       | Possible IUGR, dysmorphic features, distended abdomen, can fast for only 4–5 h after which the glucose levels drop to 40 s, insulin 1 μU/mL (ref 3–19), renal tubular acidosis type 4, only one kidney, bilateral club feet, congenital hydrocephalus not requiring shunt; c.2971C>T, p.Leu991Ile |
| 3       | 1 yr  | F             |        | IUGR, low glucose fasting (35–67 mg/dL), seizures; previous testing found “regions of homozygosity” in INSR region by SNP array |
| 4       | 5 yr  | Multi-ethnicity | M       | Delivered at 27 weeks and has complications of prematurity, holoprosencephaly, absence of corpus; loss of white matter on both occipital lobes, FTT, insulin 379.6 μU/mL (ref 3–17) |
| 5       | 11 yr | F             |        | Hypertriglyceridemia, low HDL cholesterol, high LDL cholesterol, nonalcoholic steatohepatitis, acanthosis nigricans, fasting glucose normal, insulin 70.5 μU/mL (ref 3–12) |
| 6       | 15 yr | NA            | M       | Extreme insulin resistance type A |
| 7       | 15 yr | African-American | F       | Acanthosis nigricans, amenorrhea, insulin 21 μU/mL (ref 3–19) |
| 8       | 16 yr | African-American | M       | IUGR, FTT, dysmorphic features, lack of subcutaneous fat, poor response to exogenous insulin or hyperforin |
| 9       | 21 yr | African-American | F       | Acanthosis nigricans, cystic ovaries, insulin 65.8 μU/mL (ref 2.6–24.9), severe insulin resistance, cystic ovaries. Medications: Trajenta, metformin, Depo-Provera therapy |
| 10      | 28 yr | Caucasian     | F       | Cystic ovaries, glucose fasting 89 mg/dL, hx of heavy irregular periods, mental health symptoms, cystic ovaries |
| 11      | 29 yr | Asian Indian  | F       | Amenorrhea, cystic ovaries |
| 12      | 30 yr | Caucasian     | F       | Cystic ovaries |
| 13      | 50 yr | NA            | F       | Glucose fasting, 277 mg/dL (ref 70–99) unknown if fasting, insulin antibody 1.9 U/mL (<0.4), triglyceride 1302 mg/dL (ref 40–149); cholesterol 231 mg/dL (ref 120–199) |
| 14      | 66 yr | Caucasian     | F       | Aggression, hyper-androgenism, gingival hyperplasia, thick skin, amenorrhea, distended abdomen, reported high fasting glucose fasting, high postprandial glucose 1501 mg/dL (ref <180 mg/dL) |

a Within normal reference range.
b Patient from Haiti, ethnicity unknown.
c Patient from Puerto Rico.
4. Discussion

Mutations in \textit{INSR} can cause the insulin-resistant syndromes Leprechaunism, Rabson–Mendenhall syndrome, and type A insulin resistance \cite{5,6}. Diagnosis is established on clinical examination as well as laboratory diagnostic tests with markedly elevated insulin levels being a constant feature. Functional studies (insulin binding to cultured fibroblasts) and DNA analysis can be used for definitive confirmation, keeping in mind that certain mutations do not decrease insulin binding and that DNA analysis is still not identifying all putative mutations. Although there is no straightforward genotype–phenotype correlation, mutations affecting the alpha subunit of the receptor are associated with a more severe phenotype than the mutations affecting the beta subunit \cite{25}.

Due to the lack of a central repository of \textit{INSR} mutations, we compiled a list of the published mutations, using currently accepted standards (Table 4). Our literature search of \textit{INSR} mutations identified 132 causative variations. The vast majority of these variations are missense and nonsense mutations (78\%) (Fig. 1). Interestingly, different missense mutations in the same codon have been reported to produce different phenotypes (Table 4). This highlights the need to expand the currently available databases to allow better understanding of the genotype–phenotype correlation.

There are five reports of large deletions within the \textit{INSR} gene including an entire gene deletion \cite{26}. Gross deletions and gene rearrangements account for about 5\% of the mutations \cite{26}. Large deletions, as in one of our patients, can be detected by CGH/SNP arrays. For this reason, development of a commercial test to detect single exon and whole gene deletions may be attractive. No commercial deletion/duplication testing is currently available in the US; however, deletion and duplication testing is offered at laboratories in the United Kingdom and Germany. A multiplex ligation dependent probe amplification (MLPA) assay could be used to detect single exon deletions in the \textit{INSR} gene.

DNA sequencing can identify novel sequence variants of unknown clinical significance. In our study, we detected a novel c.425G→T, p. Gly142Val affecting the insulin binding alpha subunit of the insulin receptor. The evolutionary conservation analysis by Polyphen and SIFT predicts that a glycine to valine amino acid change at this position is “possibly damaging” or “damaging” to the function of the protein. Cells from this patient (TGB) failed to bind insulin, supporting a damaging role of the identified mutation. A second mutation in this patient could not be detected indicating the limitations of the current test in detecting mutations in the deep intronic or promoter regions or deletions, duplications, and rearrangements of the gene. In fact, sequencing failed to identify the second mutation in three patients with markedly reduced insulin binding in which previous studies also failed to detect the second pathogenic change \cite{6,13}.

An additional sample of a pediatric patient referred for possible Leprechaunism was an apparent homozygous for c.2971C>A, p. Leu991Ile. A review of clinical data indicated normal to low insulin levels, a possibly damaging to the function of the protein. Cells from this patient (TGB) failed to bind insulin, supporting a damaging role of the identified mutation. A second mutation in this patient could not be detected indicating the limitations of the current test in detecting mutations in the deep intronic or promoter regions or deletions, duplications, and rearrangements of the gene. In fact, sequencing failed to identify the second mutation in three patients with markedly reduced insulin binding in which previous studies also failed to detect the second pathogenic change \cite{6,13}.

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Association studies show a strong correlation between single nucleotide polymorphism (SNP) in the \textit{INSR} gene and a predisposition to type 2 diabetes \cite{27}. An alternative isoform of exon 8 in the \textit{INSR} gene in the Han population confers increased risk for central obesity, hypertension, glucose intolerance, hyperinsulinemia and type 2 diabetes \cite{28}, whereas variation in exon 17 is associated with insulin resistance, hyperandrogenism and polycystic ovarian syndrome (PCOS) \cite{29}.

In conclusion, we report the development of a sequencing assay to detect mutations within the coding region and intron/exon boundaries of the \textit{INSR} gene. Further development of deletion/duplication analysis is needed to detect deletions, duplications and large gene rearrangement of the \textit{INSR} gene. A compilation

at position L991 to the branched amino acid isoleucine may result in steric hindrance and decreased transporter activity. In light of the fact that the patient has only one kidney and is hypoglycemic, sequencing of \textit{HNF1B} in this patient may be appropriate.
Table 4
Compilation of reported INSR mutations.

| Location | Mutation type | Nucleotide change | Amino acid change (HGVS nomenclature) | Amino acid change (legacy, mature protein) | Phenotype | Reference |
|----------|---------------|------------------|--------------------------------------|------------------------------------------|-----------|-----------|
| Exon 1   | Nonsense      | c.90C>T          | p.Tyr30Term                          | Tyr3Term                                 | Rabson–Mendenhall syndrome | [30]      |
| Exon 2   | Missense      | c.121C>T         | p.Arg41Trp                           | Arg14Trp                                 | Rabson–Mendenhall syndrome | [31]      |
| Exon 2   | Missense      | c.257A>G         | p.Asp86Gly                           | Asp59Gly                                  | Insulin resistance     | [35]      |
| Exon 2   | Missense      | c.338G>C         | p.Arg113Pro                          | Arg86Pro                                  | Insulin resistance     | [36]      |
| Exon 2   | Missense      | c.359T>A         | p.Leu120Gln                          | Leu93Gln                                  | Insulin resistance     | [37]      |
| Exon 2   | Missense      | c.433C>T         | p.Arg145Cys                          | Arg118Cys                                 | Insulin resistance A   | [38]      |
| Exon 2   | Missense      | c.479G>A         | p.Trp13Term                          | Trp118Term                                | Leprechaunism         | [40]      |
| Exon 2   | Missense      | c.489G>T         | p.Val167Leu                          | Val140Leu                                 | Insulin resistance A   | [41]      |
| Exon 2   | Missense      | c.511T>A         | p.Ile172Ser                          | Ile145Ser                                 | Diabetes, NIDDM       | [42]      |
| Exon 2   | Missense      | c.557G>T         | p.Cys186Phe                          | Cys159Phe                                 | Rabson–Mendenhall syndrome | [19]      |
| Exon 2   | Missense      | c.586T>A         | p.Cys196Ser                          | Cys169Ser                                 | Diabetes, NIDDM       | [42]      |
| Exon 3   | Missense      | c.707A>G         | p.His236Arg                          | His209Arg                                 | Leprechaunism         | [42]      |
| Exon 3   | Missense      | c.712G>A         | p.Glu238Lys                          | Glu211Lys                                 | Rabson–Mendenhall syndrome | [30]      |
| Exon 3   | Missense      | c.766C>T         | p.Arg256Cys                          | Arg229Cys                                 | Rabson–Mendenhall syndrome | [19]      |
| Exon 3   | Missense      | c.779T>C         | p.Leu260Pro                          | Leu233Pro                                 | Insulin resistance     | [44]      |
| Exon 3   | Missense      | c.839G>A         | p.Cys280Tyr                          | Cys253Tyr                                 | Insulin resistance A   | [46]      |
| Exon 3   | Missense      | c.902G>A         | p.Cys301Tyr                          | Cys274Tyr                                 | Rabson–Mendenhall syndrome | [19]      |
| Exon 4   | Missense      | c.1049C>T        | p.Ser350Leu                          | Ser323Leu                                 | Insulin resistance     | [50]      |
| Exon 4   | Nonsense      | c.1072C>T        | p.Arg358Term                         | Arg331Term                                | Insulin resistance     | [51]      |
| Exon 4   | Nonsense      | c.1114C>T        | p.Arg372Term                         | Arg345Term                                | Insulin resistance A   | [52]      |
| Exon 5   | Nonsense      | c.1156G>A        | p.Gly386Ser                          | Gly359Ser                                 | Rabson–Mendenhall syndrome | [53]      |
| Exon 5   | Missense      | c.1177G>A        | p.Gly393Arg                          | Gly366Arg                                 | Leprechaunism         | [54]      |
| Exon 5   | Missense      | c.1195C>T        | p.Arg399Term                         | Arg372Term                                | Insulin resistance     | [55]      |
| Exon 5   | Nonsense      | c.1225T>G        | p.Phe409Val                          | Phe382Val                                 | Insulin resistance     | [56]      |
| Exon 5   | Missense      | c.1246C>T        | p.Arg416Term                         | Arg389Term                                | Leprechaunism         | [57]      |
| Exon 5   | Missense      | c.1316G>C        | p.Trp439Ser                          | Trp412Ser                                 | Rabson–Mendenhall syndrome | [58]      |
| Exon 5   | Missense      | c.1372A>G        | p.Asn458Asp                          | Asn431Asp                                 | Insulin resistance     | [37]      |
| Exon 5   | Missense      | c.1459A>G        | p.Lys487Glu                          | Lys460Glu                                 | Leprechaunism         | [59]      |
| Exon 5   | Missense      | c.1466A>G        | p.Asn489Ser                          | Asn462Ser                                 | Insulin resistance     | [32]      |
| Exon 8   | Missense      | c.1627A>T        | p.Thr543Ser                          | Thr516Ser                                 | Diabetes, NIDDM       | [42]      |
| Location | Mutation type | Nucleotide change | Amino acid change (HGVS nomenclature) | Amino acid change (legacy, mature protein) | Phenotype | Reference |
|----------|---------------|-------------------|--------------------------------------|------------------------------------------|-----------|-----------|
| Exon 8   | Missense      | c.1650G>A         | p.Ala550Ala                          | Ala523Ala                                | Association with reduced diastolic blood pressure | [60]      |
| Exon 9   | Missense      | c.1975T>C         | p.Trp659Arg                          | Trp632Arg                                | Leprechaunism | [61]      |
| Exon 10  | Nonsense      | c.2095C>T         | p.Gln699Term                         | Gln672Term                               | Leprechaunism | [59]      |
| Exon 10  | Missense      | c.2201A>C         | p.Asp734Ala                          | Asp707Ala                                | Leprechaunism | [62]      |
| Exon 12  | Missense      | c.2286G>T         | p.Arg762Ser                          | Arg735Ser                                | Insulin resistance | [63] |
| Exon 12  | Nonsense      | c.2437C>T         | p.Arg813Term                         | Arg786Term                               | Leprechaunism | [64]      |
| Exon 12  | Missense      | c.2453A>C         | p.Tyr818Cys                          | Tyr791Cys                                | Leprechaunism | [65]      |
| Exon 13  | Missense      | c.2572A>G         | p.Ala858Ala                          | Thr831Ala                                | Diabetes, NIDDM | [66]      |
| Exon 13  | Missense      | c.2621C>T         | p.Ala879Ala                          | Thr858Ala                                | Leprechaunism/ Rabson–Mendenhall syndrome | [31]      |
| Exon 13  | Nonsense      | c.2668C>T         | p.Arg907Term                         | Arg880Term                               | Leprechaunism | [65]      |
| Exon 13  | Missense      | c.2696G>C         | p.Arg900Pro                          | Arg863Pro                                | Diabetes, NIDDM | [42]      |
| Exon 13  | Nonsense      | c.2673T>A         | p.Tyr891Term                         | Tyr864Term                               | Insulin resistance A | [46] |
| Exon 14  | Missense      | c.2717C>G         | p.Ala906Gly                          | Ala879Gly                                | Diabetes, NIDDM | [42]      |
| Exon 14  | Nonsense      | c.2770C>T         | p.Arg924Term                         | Arg897Term                               | Leprechaunism | [23]      |
| Exon 14  | Missense      | c.2774T>C         | p.Ile925Thr                          | Ile898Thr                                | Leprechaunism | [6]       |
| Exon 14  | Missense      | c.2776C>T         | p.Arg926Trp                          | Arg899Trp                                | Leprechaunism | [6]       |
| Exon 15  | Missense      | c.2810C>T         | p.Arg924Trp                          | Arg897Trp                                | Rabson–Mendenhall syndrome | [23] |
| Exon 15  | Nonsense      | c.2971C>T         | p.Leu991Ile                          | Leu964Ile                                | Rabson–Mendenhall syndrome | [6] |
| Exon 16  | Missense      | c.2989C>T         | p.Pro997Thr                          | Pro970Thr                                | Rabson–Mendenhall syndrome | [6] |
| Exon 17  | Missense      | c.3034G>A         | p.Val1012Met                         | Val985Met                                | Diabetes, NIDDM | [68]      |
| Exon 17  | Missense      | c.3059G>A         | p.Arg1020Gln                         | Arg993Gln                                | Insulin resistance | [69] |
| Exon 17  | Nonsense      | c.3077G>T         | p.Ile925Thr                          | Ile898Thr                                | Insulin resistance | [70] |
| Exon 17  | Missense      | c.3104G>A         | p.Gly1054Val                         | Gly1028Val                               | Diabetes, NIDDM | [14]      |
| Exon 17  | Nonsense      | c.3160C>T         | p.Arg1027Term                        | Arg1000Term                              | Insulin resistance | [32] |
| Exon 17  | Missense      | c.3164C>T         | p.Arg1054Met                         | Arg1027Met                               | Rabson–Mendenhall syndrome | [61] |
| Exon 17  | Nonsense      | c.3224C>A         | p.Val1055Val                         | Val1028Val                               | Insulin resistance A | [41] |
| Exon 17  | Missense      | c.3255C>T         | p.Arg1024Asp                         | Arg1028Asp                               | Insulin resistance | [72] |
| Exon 17  | Nonsense      | c.3257T>A         | p.Arg1054Met                         | Arg1027Met                               | Rabson–Mendenhall syndrome | [13] |
| Exon 17  | Missense      | c.3355C>T         | p.Arg1119Gln                         | Arg1092Gln                               | Rabson–Mendenhall syndrome | [11] |
| Exon 18  | Nonsense      | c.3428T>C         | p.Arg1158Trp                         | Arg1131Trp                               | Rabson–Mendenhall syndrome | [49] |
| Exon 19  | Missense      | c.3463G>C         | p.Ala1161Thr                         | Ala1134Thr                               | Rabson–Mendenhall syndrome | [13] |
| Exon 19  | Missense      | c.3499A>T         | p.Ala1162Glu                         | Ala1135Glu                               | Rabson–Mendenhall syndrome | [76] |
| Exon 19  | Missense      | c.3540G>A         | p.Ala1162Glu                         | Ala1135Glu                               | Rabson–Mendenhall syndrome | [77] |
| Exon 19  | Missense      | c.3572G>A         | p.Arg1191Gln                         | Arg1164Gln                               | Diabetes, NIDDM | [79]      |
| Exon 19  | Missense      | c.3600G>A         | p.Arg1201Gln                         | Arg1174Gln                               | Insulin resistance | [80] |

(continued on next page)
of all the mutations reported to date using current terminology (Table 4) is the first step toward development of a publicly available online mutation database for the \textit{INSR} gene.

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### Table 4 (continued)

**A. Missense/nonsense mutations**

| Location | Mutation type | Nucleotide change (HGVS nomenclature) | Amino acid change (legacy, mature protein) | Phenotype | Reference |
|----------|---------------|---------------------------------------|-------------------------------------------|-----------|-----------|
| Exon 20  | Missense      | c.3601C>T p.Arg1201Trp                | Arg1174Trp                                | Leprechaunism | [81]      |
| Exon 20  | Missense      | c.3614C>T p.Pro1205Leu                | Pro1178Leu                                | Insulin resistance | [82]      |
| Exon 20  | Missense      | c.3618G>A p.Glu1206Asp                | Glu1179Asp                                | Insulin resistance | [83]      |
| Exon 20  | Missense      | c.3616G>A p.Glu1206Lys                | Glu1179Lys                                | Leprechaunism | [49]      |
| Exon 20  | Missense      | c.3659G>T p.Trp1220Leu                | Trp1193Leu                                | Insulin resistance | [83]      |
| Exon 21  | Missense      | c.3680G>C p.Trp1227Ser                | Trp1200Ser                                | Insulin resistance | [76]      |
| Exon 21  | Nonsense      | c.3769C>T p.Gln1257Term               | Gln1230Term                               | Insulin resistance | [73]      |
| Exon 22  | Missense      | c.4082A>G p.Tyr1361Cys                | Tyr1334Cys                                | Diabetes, NIDDM   | [66]      |
| Exon 22  | Missense      | c.4133G>A p.Arg1378Gln                | Arg1351Gln                                | Insulin resistance | [50]      |

**B. Splice site, insertion/deletion, and large gene rearrangement mutations**

| Location | Mutation type | Nucleotide change | Phenotype | Reference |
|----------|---------------|-------------------|-----------|-----------|
| Intron 2 | Splice site   | c.1124-2A>G       | Insulin resistance | [67]      |
| Intron 5 | Splice site   | c.1268 + 2T>C     | Rabson–Mendenhall syndrome | [31]      |
| Intron 6 | Splice site   | c.1483 + 43G>T    | Diabetes, type 2, association with | [84]      |
| Intron 13| Splice site   | c.2682 + 1G>A     | Leprechaunism | [9]       |
| Intron 14| Splice site   | c.2842 + 1G>A     | Insulin resistance A | [85]      |
| Exon 17  | Splice site   | c.3258G>A         | Fiber-type disproportion myopathy, congenital | [86]      |
| Intron 21| Splice site   | c.3794 + 1G>T     | Leprechaunism | [31]      |
| Exon 1   | Deletion      | c.22_31del10      | Insulin resistance A | [41]      |
| Exon 2   | Deletion      | c.404delA         | Leprechaunism | [47]      |
| Exon 2   | Deletion      | c.444_446delGAA   | Leprechaunism | [88]      |
| Exon 3   | Deletion      | c.927_929delICAA  | Leprechaunism | [9]       |
| Exon 4   | Deletion      | c.1084_1086delGTC | Leprechaunism | [89]      |
| Exon 9   | Deletion      | c.1998_2001delTGAG| Leprechaunism | [6]       |
| Exon 12  | Deletion      | c.2480_2487del8   | Insulin resistance | [67]      |
| Exon 15  | Deletion      | c.2944_2945delAG  | Leprechaunism | [90]      |
| Exon 17  | Deletion      | c.3077_3079delTTC | Insulin resistance | [91]      |
| Exon 19  | Deletion      | c.3408delG        | Leprechaunism | [67]      |
| Intron 20| Deletion      | c.3659 + 1_3659 + 3delGTG | Insulin resistance | [92]      |
| Exon 3   | Insertion     | c.866_867ins12    | Rabson–Mendenhall syndrome | [36]      |
| Exon 10  | Insertion     | c.2050_2051insG   | Leprechaunism | [6]       |
| Exon 2   | Large deletion| ex. 2 (c.101-652) | Insulin resistance | [70]      |
| Exon 3   | Large deletion| ex. 3 (c.653-974) | Insulin resistance | [93]      |
| Exons 10–13| Large deletion| >12 kb incl. ex. 10-13 | Leprechaunism | [49]      |
| Exon 14  | Large deletion| 1.2 kb incl. ex. 14 | Acanthosis nigricans | [94]      |
| Full gene| Large deletion| Entire gene       | Leprechaunism | [26]      |
| Exon 13  | Indel         | c.2630_2642delins5| Leprechaunism | [95]      |
| Exon 14  | Indel         | c.2752_2753delinsTG| Diabetes, NIDDM   | [42]      |
| Complex  | Indel         | Rec. \textit{INSR}/Alu | Acanthosis nigricans, insulin related | [96]      |
| Complex  | Translocation | t(7;19)(p15.2;p13.2) | Insulin resistance | [97]      |
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