Note

A Nonsense Mutation in the Arg345 of the Insulin Receptor Gene in a Japanese Type A Insulin-resistant Patient

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Abstract. Defects in insulin receptor function have been associated with insulin resistant states such as obesity and type 2 diabetes mellitus. Several types of mutations in the insulin receptor gene have been identified in patients with genetic syndromes of extreme insulin resistance. We have studied a 10-year-old Japanese girl with type A insulin resistance with hirsutism and hyperinsulinemia but without the dysmorphic features characteristic of leprechaunism or Rabson-Mendenhall syndrome. Despite the presence of severe insulin resistance, the patient did not develop overt diabetes mellitus at the time of investigation. Using direct sequencing, we identified a nonsense mutation causing premature termination after amino acid 345 in the α subunit of the insulin receptor.

Key words: Insulin receptor abnormality, Insulin resistance

THE human insulin receptor is encoded by a single gene with 22 exons and is an assembly of a disulfide bond-linked tetramer composed of two α and two β subunits [1–5]. After binding of insulin to the extracellular α subunit, the tyrosine kinase of the membrane-spanning β subunit is activated and the receptor is autophosphorylated [6]. Insulin receptor kinase regulates the action of insulin on metabolism and growth through signal transduction pathways and is therefore thought to be central to insulin action [7].

The cloning and sequencing of insulin receptor cDNA have made it possible to identify some dozens of mutations in the human insulin receptor gene to date [8–12]. We need to precisely diagnose this disease at the genetic level for the appropriate selection of treatment regimen and the prediction and prevention of development of diabetes in their family members.

In this study, we identified a heterozygous mutation causing premature termination at amino acid 345 substituting a termination codon for arginine in the L2 domain in α subunit of the insulin receptor gene in a Japanese patient with hyperinsulinemia, insulin resistance, and mild glucose intolerance.

Subjects and Methods

Patient

The proband, a girl of 10 years old, presented with symptoms of general malaise and loss of appetite. She was born to unrelated Japanese parents who were normal from a clinical point of view at the time of investigation. Her mother showed abnormal glucose tolerance during pregnancy and her maternal grandfather had suffered from diabetes mellitus. Laboratory data or specimens from the patient’s family were not available for study. At birth, she did not have the dysmorphic features characteristic of leprechaunism or
Rabson-Mendenhall syndrome, including intrauterine growth retardation, fasting hypoglycemia, and abnormalities of teeth and nails. At presentation, she was thin, pale and showed hirsutism but no pigmentation including acanthosis nigricans. She lost 6 kg in the two to three months before she visited the hospital. Because urinalysis revealed strongly positive ketone reactivity, she was hospitalized for whole body control and further evaluation.

The study was approved by the Ethical Committee of Ehime University School of Medicine and the investigation was carried out in accordance with the guidelines in the Declaration of Helsinki. Written informed consent was obtained from the patient and her parents.

Amplification of genomic DNA

Genomic DNA was extracted from the patient’s leukocytes using a DNA isolation kit for mammalian blood (Boehringer Mannheim Corp., Indianapolis, IN, USA). Exons 1-22 of the insulin receptor gene were individually amplified using primer sets as described [13]. One μg of genomic DNA was amplified using 2.5 U of Thermus aquaticus (Taq) DNA polymerase (Takara Co., Ltd. Biomedical Group, Shiga, Japan), 20 pmol of each primer, and 20 nmol of each dNTP. After the first denaturing for 3 min at 94°C, PCR was carried out for 30 cycles at 94°C for 30 sec, at 55°C for 30 sec, and at 72°C for 1 min with a final extension for 3 min. Amplified PCR products were electrophoresed on an agarose gel and then stained with cyber green to confirm the size of each fragment.

Direct sequencing of amplified genomic DNA

These PCR products were purified by SUPREC™-02 (Takara), followed by sequencing using forward and reverse primers as described [14]. The sequencing reaction was carried out using Taq Dye Deoxy™ and ABI Prism™ terminator cycle sequencing kits (Applied Biosystems, Foster City, CA, USA). The products were then purified on a Centri-Sep column (Princeton Separations, Inc., Adelphia, NJ, USA), dried on a speed vacuum centrifuge, and resuspended in template suppression reagent (Applied Biosystems). After denaturing at 95°C for 3 min, the products were quickly placed on ice for 10 min and then electrophoresed on an ABI gene analyzer 310 system (Applied Biosystems).

Results

Examinations of the patient revealed the presence of extreme insulin resistance (Table 1). Severe fasting hyperinsulinemia was reproducibly observed and homeostasis model assessment of insulin resistance (HOMA-IR) [15], calculated from data obtained by 75-g oral glucose tolerance tests (OGTT), was 7.58 and 15.84, respectively. Two insulin tolerance tests (0.1 and 0.17 U/kg), performed on separate dates, resulted in a 26.0% and an 18.2% reduction in plasma glucose, respectively. All these findings were indicative of insulin resistance in our patient. Since the patient’s fibroblasts and/or erythrocytes were not available, we could not determine the insulin binding activity of the patient’s insulin receptors. Therefore, we provisionally diagnosed the patient as having type A insulin resistance in the broad sense of the term, based on the absence of autoantibody against insulin receptor.

Laboratory findings of the patient on admission, summarized in Table 1, showed normal levels of HbA1c and fasting plasma glucose. Although a 75-g OGTT conducted just after the admission demonstrated diabetes pattern with plasma glucose level of 228 mg/dl two hours after the glucose loading, the patient demonstrated impaired glucose tolerance pattern in the other 75-g OGTT conducted prior to discharge, which showed a plasma glucose level of 142 mg/dl two hours after the glucose loading. During her hospital stay, no intensive alimentary therapy or insulin therapy was employed and the patient was only subjected to intravenous drip of maintenance infusion in the first 5 days of the hospitalization. Thus, the patient gradually restored her general condition by supportive care without medication and was discharged from the hospital in two weeks. We therefore concluded that the patient did not develop diabetes mellitus in spite of the fact that she showed diabetes pattern in the initial OGTT (see Discussion).

Sequencing analysis of the 22 exons as well as the intron-exon junctions containing consensus sequences

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1The numbering of insulin receptor nucleotides and amino acids used in this paper was according to Ullrich et al. [4].
required for RNA splicing of the insulin receptor identified a heterozygous mutation at nucleotide position 1162 substituting a termination codon for arginine 345, a conserved amino acid in insulin-like growth factor I receptor and insulin receptor-related receptor [16], in the putative receptor L2 domain in exon 4 of the patient’s insulin receptor (Fig. 1). No other mutations were found in the entire insulin receptor sequence analyzed in this study.

Discussion

The major finding of this article is the identification, in a Japanese patient with hyperinsulinemia and severe insulin resistance, of a previously undescribed heterozygous mutation in the patient’s insulin receptor. We conjectured that the patient did not develop overt diabetes mellitus, based on the following reasons. First, careful history examination revealed that she might have been starving over an extended period of time owing to loss of appetite before hospitalization. This was followed by starvation ketosis, as was suggested by strongly positive ketone reactivity in her urinary examination at the time of admission. It is likely
that glucose tolerance was impaired under such conditions [17]. Second, during the subsequent outpatient follow-up for 3 years, she consistently showed hyperinsulinemia but did not develop diabetes mellitus up to the present date.

We identified a nonsense mutation in one allele of a patient substituting the termination codon (TGA) for the CGA codon normally encoding Arg\textsuperscript{345} located in the putative L2 domain, which consists of a single-stranded right-hand beta-helix and is suggested to make up the bilobal ligand binding site [18–20]. The CGA (arginine) codon has previously been identified as a “hot spot” for mutations [21]. It was presumed that the CpG sequence is a substrate for methylation and 5-methylcytosine gives propensity to DNA replication errors, converting C·G base pair to a T·A base pair [21]. The nonsense mutation at codon 345 truncated C-terminal half of the receptor α subunit as well as the entire β subunit including the transmembrane anchor and the tyrosine kinase domain. Thus, it is unlikely that this truncated receptor, translated from the mutant allele, would be functional or located on the cell surface.

Nonsense mutations were frequently associated with reduction of steady-state mRNA levels by the premature insertion of a termination codon not only in the insulin receptor gene [22, 23] but also in a number of genes [24–27]. In fact, reduced insulin-receptor mRNA was found in a female leprechaunism patient of Hispanic and Afro-American descent bearing a nonsense mutation in the L2 domain at codon 372, a position in close proximity to Arg\textsuperscript{345}, substituting the termination codon for Arg\textsuperscript{372} of the patient’s insulin receptor [28]. She was suggested to be a compound heterozygote, based on the biochemical phenotype of her insulin receptor, and presented a severe phenotype of leprechaunism with extreme insulin resistance and a number of congenital abnormalities. The milder clinical characteristics observed in our patient might be attributable to the fact that our patient was not a compound heterozygote but was heterozygous for the mutation at nucleotide position 1162, hence the overall insulin receptor mRNA level was not so remarkably reduced due to the presence of the functional receptor translated from the normal allele.

The existence of mutations that cause less severe impairment of receptor function and the difficulty in detecting such mutations had already been predicted earlier by Taylor et al. [8]. Advances in the technology to determine nucleotide sequences have made it easier to find insulin receptor abnormalities in patients who have only mild impairment in their glucose tolerance, as was shown in this study. In this sense, we need to bear in mind the likelihood that such mutations may be present in patients with severe hyperinsulinemia and less severe glucose intolerance.

Mutations of the insulin receptor gene can be classified according to their biochemical phenotype in the context of the life cycle of the insulin receptor [29]. Thus, mutations of the insulin receptor thus far reported have been classified into five classes: class I in which impaired receptor biosynthesis was found; class II with impaired intracellular processing and transport to the plasma membrane; class III showing decreased affinity of insulin binding; class IV comprising mutations which impair receptor autophosphorylation and kinase activity; and class V showing decreased receptor recycling through defects in acid-dependent dissociation of receptor and its ligand in early endosome, followed by accelerated receptor degradation in lysosome. In this context, our patient may be categorized in class I according to the above criteria.

In conclusion, we identified a nonsense mutation causing premature termination after amino acid 345 in the α subunit of the insulin receptor in a Japanese insulin resistant patient. Further investigations including determination of mRNA level as well as ligand binding and receptor autophosphorylation would be required to address the molecular mechanism by which this mutation leads to the insulin resistance observed in our patient.

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