Phenotypical variety of insulin resistance in a family with a novel mutation of the insulin receptor gene

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Abstract. A novel mutation of insulin receptor gene (INSR gene) was identified in a three generation family with phenotypical variety. Proband was a 12-year-old Japanese girl with type A insulin resistance. She showed diabetes mellitus with severe acanthosis nigricans and hyperinsulinemia without obesity. Using direct sequencing, a heterozygous nonsense mutation causing premature termination at amino acid 331 in the α subunit of INSR gene (R331X) was identified. Her father, 40 years old, was not obese but showed impaired glucose tolerance. Her paternal grandmother, 66 years old, has been suffered from diabetes mellitus for 15 years. Interestingly, they had the same mutation. One case of leprechaunism bearing homozygous mutation at codon 331 was identified. These findings led to the hypothesis that R331X may contribute to the variation of DM in the general population in Japan. An extensive search was done in 272 participants in a group medical examination that included 92 healthy cases of normoglycemia and 180 cases already diagnosed type 2 DM or detected hyperglycemia. The search, however, failed to detect any R331X mutation in this local population. In addition, the proband showed low level C-peptide/insulin molar ratio, indicating that this ratio is considered to be a useful index for identifying patients with genetic insulin resistance. In conclusion, a nonsense mutation causing premature termination after amino acid 331 in the α subunit of the insulin receptor was identified in Japanese diabetes patients. Further investigations are called for to address the molecular mechanism.

Key words: Insulin receptor, Insulin resistance, Type 2 diabetes, Leprechaunism, C-peptide/insulin molar ratio

THE INTERACTION of insulin with its cell surface receptor is the first step in insulin action and the first identified target of insulin resistance. Mutations in the insulin receptor gene lead to the insulin resistance in several syndromic forms. 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].

Some dozens of mutations in the human insulin receptor gene have already been identified to date [8-11]. Homozygous or compound-heterozygous mutations in the insulin receptor gene are found in patients with syndromes of severe insulin resistance [12]. More severe Donohue syndrome (“Leprechaunism” OMIM 246200) and the milder Rabson-Mendenhall syndrome (OMIM 262190) are characterized by intrauterine and postnatal growth retardation, facial dysmorphism, lack of subcutaneous fat and altered glucose homeostasis with hyperinsulinemia, acanthosis nigricans and reduced life expectancy [13-15]. Cells from most patients with Donohue syndrome show absent or
severely reduced insulin binding, whereas those with Rabson-Mendenhall retain some insulin binding capacity. Therefore, it has been proposed that severity of the phenotype is determined by the degree of insulin resistance and that residual insulin binding capacity correlates with survival. Heterozygous mutations in the insulin receptor gene have been demonstrated in type A insulin resistance with the triad of insulin resistance, acanthosis nigricans, and hyperandrogenism (OMIM147670) [16].

In this study, we identified a heterozygous mutation causing premature termination at amino acid 331 substituting a termination codon for arginine in the L2 domain in α subunit of the insulin receptor gene in a Japanese patient with diabetes mellitus and hyperinsulinemia. Interestingly, her family members shared the same mutation but showed different clinical course.

Materials and Methods

Subjects

The proband, a girl of 12 years old, was referred to our hospital because of glucosuria detected by school urinary screening. She presented with mild symptoms of polydipsia and polyuria. She was born to unrelated Japanese parents at 37 weeks of gestation (birth weight 2495 g, birth length 48 cm). At birth, she did not have the dysmorphic features characteristic of leprechaunism or Rabson-Mendenhall syndrome, including intrauterine growth retardation, fasting hypoglycemia. Sensorineural hearing loss in right side was diagnosed when she was infant, but did not deteriorate.

At presentation, she was not obese, but showed severe acanthosis nigricans with scratching scar of her neck. It also mildly existed at the axilla and elbow. Hirsutism was not observed. Body mass index (BMI) was 21.6 (height 148.6 cm, weight 47.7 kg). Blood pressure was 110/70 mmHg. Pubertal stage was B2 and PH1. Laboratory tests revealed the following; HbA1c, 9.2 %; FPG, 124 mg/dL; IRI, 65.7 µU/mL; C-peptide, 3.18 ng/mL; AST, 20 IU/L; ALT, 18 IU/L; total cholesterol, 194 mg/dL; HDL cholesterol, 43.7 mg/dL; testosterone, 0.33 ng/mL. Islet associated autoantibodies were absent. Urine testing showed no ketonuria but proteinuria (microalbumin 64.4 mg/g cr) and glucosuria. Ocular complication and retinopathy was not detected. Abdominal CT revealed no fatty liver and area of visceral fat on umbilical level was 41.8 cm² (normal: 60+). Although she showed diabetes mellitus with severe insulin resistance, her data of body composition was not suggested risk for obesity or metabolic syndrome. Self monitored blood glucose levels were 120-140 mg/dL at premeal time and 170-200 mg/dL at postprandial time. Her father, 40 years old, was healthy and no obesity (BMI 21.8) from a clinical point of view at the time of investigation. Her paternal grandmother, 66 years old, has been suffered from diabetes mellitus. She was also not obese (BMI 21.6) and has been treated with sulfonylureas for 15 years. She already developed retinopathy and presented vitreous hemorrhage 10 years ago. Her younger brother, seven years of age, had mild mental retardation and supported by special education. He showed mild obesity but normal response to oral glucose tolerance test without hyperinsulinemia (FPG, 86 mg/dL; IRI, 8.6 µU/mL; C-peptide, 1.53 ng/mL).

Measurements

The standard 75 g oral glucose tolerance test (OGTT) was performed, after overnight fast. Levels of glucose, insulin and C-peptide were measured at 0, 30, 60, 90 and 120 min. Insulin was measured using an enzyme immunoassay (E test TOSOH II; TOSOH Corporation, Tokyo, Japan). Cross-reactivity with proinsulin was 2 %. C-peptide was measured using a chemiluminescent enzyme immunoassay (LUMIPULSE Presto C-peptide; FUJIREBIO Inc., Tokyo, Japan). Proinsulin was measured using a RIA2 antibody method (HUMAN PROINSULIN RIA KIT; Linco Research Inc., St. Charles, MO).

We calculated C-peptide/insulin molar ratio from each molecular weight and international unit of insulin i.e. 26 IU/mg. We estimated molecular weight of insulin at 5800 and C-peptide at 3600. Consequently, 1 µU/mL of insulin is 6.09 pmol/L and 1 ng/mL of C-peptide is 0.278 nmol/L.

Sequence analysis

Informed consent was obtained from her family. Genomic DNA was extracted from peripheral blood lymphocytes using a DNA isolation kit for mammalian blood. Exon 1-2 of the insulin gene and Exons 1-22 of the insulin receptor gene were individually amplified using primer sets as described [17, 18]. PCR products were purified for direct sequence analysis on an ABI gene analyzer 310 or 3100 system according to the manufacturer’s instructions (Applied Biosystems).
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Analysis for prevalence of R331X mutant in population

We tested the frequency of R331X in type 2 DM or by chance hyperglycemia in adult people, living in the Akita prefecture located in northern Japan. We studied 272 participants of a group medical examination, comprised 92 healthy cases checked normoglycemia and 180 cases already diagnosed type 2 DM or detected hyperglycemia. These included 47 cases with family history of DM and 14 cases diagnosed before third decade. All participants gave informed consent, and the Ethics Committee of Kyoto University School of Medicine approved the study.

Genotyping of R331X was assayed with PCR restriction fragment length polymorphism. PCR reactions were conducted in a reaction volume of 7.5 μL with 20 ng genomic DNA, 2× GC buffer, 200 μM dNTPs, 10 pmol of each primer and 1 unit of LA Taq polymerase (Takara, Tokyo, Japan). The PCR primers used were 5’-AGATGTCTGAGGAGCTTGAAGGACCTTGGA-3’ as a forward primer and 5’-ACAGCTCAGAGGGACATGGA-3’ as a reverse primer. PCR was performed with 39 cycles of the following 94°C for 45 s, 54°C for 45 s and 74°C for 1 min in a thermocycler. Obtained PCR products showed a single fragment at 285 bp. Six μL of 285-bp product were then digested with 2 units of BspCNI restriction enzyme at 25 °C for 2 h. Digestion products were visualized on a 3 % agarose gel. Wild-type allele produced double band at 269 and 16 bp and mutant allele produced three bands at 165, 104 and 16 bp.

Results

An OGTT revealed a diabetic pattern with hyperinsulinemia (Table 1). The homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance, was 20.1. The C-peptide/insulin molar ratio was extremely low. The fasting and 120 min levels were 2.21 and 1.57, respectively (normal level of fasting is 4.0<). An insulin tolerance test (0.1U/kg insulin i.v.) showed insulin resistance with only 37 % reduction in plasma glucose levels. Metformin was started from 250 mg/day and increased up to 500 mg/day. HbA1c levels improved to 5-6 % six months later. At that point in time, her fasting proinsulin level was 71.7 pmol/L when the IRI level was 49.1 μg/mL. Proinsulin/insulin molar ratio was 0.24 (normal 0.1-0.2). Her insulin levels were still high; however, the acanthosis nigricans had disappeared after she had regained diabetic control.

Her clinical course suggested two genetic diseases of glucose metabolism. One was the insulin gene mutation, as characterized by a low level C-peptide/insulin molar ratio, and sometimes presents as type 2 DM. The other was the insulin receptor gene mutation, which clinically demonstrated type A insulin resistance.

A sequencing analysis of the 22 exons as well as the intron-exon junctions identified a heterozygous mutation at nucleotide position 1072 substituting a termination codon for arginine 331, a conserved amino acid in the insulin-like growth factor I receptor and insulin receptor-related receptor, in the putative receptor L2 domain of the patient’s insulin receptor (Fig. 1) [19]. No other mutations were found in any of the insulin receptor genes analyzed in this study.

Her father and grandmother also had the same
heterozygous mutation (data not shown). The fasting C-peptide/insulin molar ratio of her grandmother was relatively low under the treatment of sulfonylureas (3.84, IRI; 30.1 μU/mL, CPR 2.51 ng/mL). The HbA1c level of her father was 4.7 %, but OGTT showed impaired glucose tolerance (Table1). Although the fasting insulin level was 10.1 μU/mL, it increased up to 100 μU/mL in 60-120 min. The C-peptide/insulin molar ratio was 5.60 in fasting and 3.40 in 120 min. They showed milder insulin resistance in comparison to the proband. The heterozygous mutation seemed to significantly affect the insulin resistance of the three subjects, even if no typical skin lesions were observed in either the father or grandmother.

One unrelated case of leprechaunism with R331X homozygous mutation was identified in Tokyo, Japan. The patient was born to unrelated parents at 39 weeks of gestation with a birth weight of 1743 g. She showed an extreme degree of insulin resistance (FPG, 200 mg/dL<; IRI 10,000 μU/mL<). She thereafter started to receive subcutaneous injections of recombinant human IGF-I. After treatment, her glucose metabolic abnormality was improved. Informed consent was obtained from her parents for sequence analysis. Her parents had R331X heterozygous mutation. They did not demonstrate any symptoms of diabetes mellitus. Information on the glucose tolerance including OGTT was unavailable.

These findings led to the hypothesis that insulin receptor genetic variants contribute to the variation of DM in the general population in Japan. An extensive search was done in 272 participants in a group medical examination that included 92 healthy cases of normoglycemia and 180 cases already diagnosed as type 2 DM or detected hyperglycemia. The search, however, failed to detect any R331X mutation in this local population.

Discussion

Type A insulin resistance was initially characterized in young female patients with acanthosis nigricans, ovarian hyperandrogenism and virilization [20]. Over 30 mutations have so far been described in these patients, which are mainly clustered in the tyrosine kinase domain of the insulin receptor [21, 22].

A nonsense mutation was identified in one allele of a patient substituting the termination codon (TGA) for the CGA codon normally encoding Arg located in a putative L2 domain, which is a single stranded right-hand beta-helix and is suggested to make up the bilobal ligand binding site [23]. The nonsense mutation at codon 331 truncated the C-terminal half of the receptor α subunit as well as the entire β subunit including the transmembrane anchor and the tyrosine kinase domain. Therefore, it is unlikely that this truncated receptor, translated from the mutant allele, would be either functional or located on the cell surface. In fact, extreme insulin resistance was observed in a female leprechaunism patient with homozygous R331X alleles.

Hyperinsulinemia is usually considered to be the result of resistance to the physiological effects of insulin and consequent compensatory increased insulin secretion. Recently, the C-peptide/insulin ratio is widely used as a surrogate of hepatic insulin clearance for the evaluation in type 2 DM or glucose intolerance [24, 25]. This index, should clarify whether impaired hepatic insulin clearance or increased insu-
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lin secretion has a dominant effect on such patients. Insulin and C-peptide are secreted into the portal vein in a 1:1 molar ratio after β-cell stimulation by carbohydrate or other secretagogues. A large fraction of endogenous insulin is cleared by the liver, whereas C-peptide, which is cleared primarily by the kidney and has a lower metabolic clearance rate than insulin, and traverses the liver with essentially no extraction by hepatocytes [26, 27]. Diminished insulin clearance has been demonstrated to be an important underlying mechanism for the hyperinsulinemia found in various insulin-resistant conditions [28-30]. For example, to evaluate hyperinsulinemia in African Americans, at risk for type 2 DM, several studies used C-peptide/insulin molar ratio as an index of hepatic insulin clearance. African American children and adults showed lower C-peptide/insulin ratio than White Americans, thus suggesting that high insulin levels could be partly attributed to lower clearance [31, 32].

Therefore, the use of the C-peptide/insulin molar ratio reflects of hepatic insulin clearance [33]. A low C-peptide/insulin molar ratio of our patients suggests impaired hepatic insulin clearance because of, not only DM, but also abnormal insulin receptor expression in the liver. To this day, a low C-peptide/insulin molar ratio has not been substantially observed among individuals with type A insulin resistance. Two family cases with an insulin receptor gene mutation reported the presence of a low C-peptide/insulin molar ratio [34, 35]. They showed hyperinsulinemic hypoglycemia, severe insulin resistance and the C-peptide/insulin molar ratio ranged from 1.1 to 3.8.

As well as this reported cases, the molar ratio of the proband of our family was very low similar to that observed in subjects with insulin gene mutation. Previously, low C-peptide/insulin ratio was well reported to be a clinical feature of mutations in the human insulin gene causing either familial hyperinsulinemia or familial hyperproinsulinaemia. The elevated circulating IRI consisted mainly of the unprocessed mutated proinsulin, which had accumulated because of proinsulin’s relatively low clearance compared with insulin. In these subjects, proinsulin levels were tends to be extremely high, namely over three hundred pmol/L [36, 37]. Due to dramatic improvements in the assay techniques of IRI, cross-reactivity with proinsulin is normally seen at very low levels. Consequently, there have been no new reports regarding hyperproinsulinaemia with insulin gene mutations for the last decade.

Recently, the fasting proinsulin/insulin ratio is used as a marker of β-cell dysfunction. In peripheral blood, fasting proinsulin accounts for 10-20% of insulin but it may reach values as high as 50% in type 2 DM. Taura et al. evaluated the basal and dynamic proinsulin-insulin relationship to assess the β-cell function during OGTT in type 2 DM [38]. The proinsulin/insulin molar ratio was higher in type 2 DM (0.39 ± 0.05) subjects than normal (0.14 ± 0.01) and impaired glucose-tolerant (0.13 ± 0.02) subjects. In comparison to this study, the fasting proinsulin/insulin ratio of the proband, 0.20 was slightly higher than normal. It is difficult to consider that her low C-peptide/insulin molar ratio is derived from structural abnormalities in the proinsulin molecule.

We calculated the C-peptide/insulin molar ratio of several previous cases with insulin receptor gene mutation from data measured simultaneously. Severe cases, Rabson-Mendenhall syndrome or Donohue’s syndrome, showed very low level (0.69 to 1.83) [14, 39-41]. Milder cases, type A insulin resistance or DM, also showed relatively low molar ratio (1.47 to 4.26) [35, 42]. However, most previous case reports only recorded the IRI data, more investigations are needed to discuss these clinical characteristics.

Interestingly, the patient’s father did not show hyperinsulinemia while demonstrating a normal C-peptide/insulin molar ratio after fasting. However, after oral glucose ingestion, the insulin level increased 100.5 µU/mL/mL at 60 min and the molar ratio gradually decreased from 5.60 to 3.40. Meier et al. studied the C-peptide/insulin molar ratio as calculated at singular time points after oral glucose administration in non-diabetic subjects [37]. They reported that the molar ratio decreased to half level at 30 minutes and then it gradually increased up to the initial level through 120 min. In contrast to their data, the proband and her father showed a gradually decreasing pattern from 0 to 120 minutes. Receptor-mediated insulin endocytosis and degradation in hepatocyte underlie the basic mechanism of insulin clearance. Insulin is targeted for degradation after internalization, whereas the receptor recycles back to the cell surface [43]. CEACAM1, a transmembrane glycoprotein, plays a significant role in receptor-mediated insulin endocytosis [44]. In vitro studies suggest that upon its phosphorylation by the insulin receptor kinase, CEACAM1 binds indirectly to the receptor to undergo internalization in clathrin-coated vesicles as part of endocytosis complex [45].
CEACAM1 is considered to interact with two separate domains of the insulin receptor: a C-terminal for its phosphorylation, and cytoplasmic juxtamembrane domain required for internalization [46]. R331X mutant defects these important domains for endocytosis of insulin-insulin receptor complex. A reduction of endocytosis may also affect recycle of insulin receptor and may cause prolonged low hepatic extraction after glucose oral load observed in subjects having R331X mutation. Although her father showed normal data in fasting period, the oral glucose test may be a supplementary means for evaluating of insulin receptor mutant subjects.

As stated above, C-peptide is believed to be a better index of the pancreatic β-cell function than insulin because C-peptide levels are unaffected by hepatic clearance. When comparing the father’s C-peptide levels of OGTT with proband, only a slight difference was observed. This result indicates that the insulin secretory function of β-cell is not substantially different and the cause of hyperinsulinemia in the proband is dominantly affected by impaired hepatic insulin clearance. The evaluation of the C-peptide/insulin molar ratio is thus considered to be a useful index for identifying genetic insulin resistance patients. On the other hand, a mild phenotype such as that observed in her father may not be effectively evaluated by the fasting data alone.

Unrelated Japanese patients with another mutation of the insulin receptor gene have been previously reported. They showed different phenotypes: one was detected as a heterozygous mutation in type A insulin resistance, while the other was detected as a compound heterozygous mutation in leprechaunism, thus indicating that the severity of such mutations will determine the phenotype [47]. The phenotype of heterozygous R331X differed substantially among the current family members. Although the proband and her grandmother showed diabetes mellitus with insulin resistance, the difference in the age of onset was around forty years. In addition, her father did not show insulin resistance after fasting. The reason for this difference may be conditioned by heredity and environment. The lifestyle for children has changed over the last few decades in Japan. The proband often consumed high caloric foods before detecting glucosuria. Numerous genetic factors related to diabetes mellitus have also been investigated. The insulin receptor pathway plays an important role in the glucose metabolism. The phenotype of a homozygous mutation, leprechaunism, revealed this important function in humans. However, a heterozygous mutation including Type A insulin resistance shows a mild phenotype. Variance in the current family case suggests that various genetic factors may therefore have played a role in their glucose metabolism. Contrary to expectations, the hypothesis that R331X determines the phenotype for glucose tolerance in Japanese people was ruled out. In addition, the influence of other reported mutations was unclear.

In conclusion, a nonsense mutation causing premature termination after amino acid 331 in the α-subunit of the insulin receptor was identified in Japanese diabetes patients. The phenotype of R331X showed variety, and therefore further investigations, including determination of the mRNA level as well as ligand binding and receptor autophosphorylation, are thus called for to address the molecular mechanism by which this mutation leads to the occurrence of diabetes, as was observed in the current patient. In addition, the C-peptide/insulin molar ratio is considered to be a useful index for identifying genetic insulin resistance patients.

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References


