Hyperinsulinemic hypoglycemia syndrome associated with mutations in the human insulin receptor gene: Report of two cases

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Abstract. Insulinoma and insulin or insulin receptor (IR) autoantibodies are the main causes of hyperinsulinemic hypoglycemia in adults, but the exact cause in other cases remains obscure. This study is to determine the genetic basis of hyperinsulinemic hypoglycemia in two cases without the above abnormalities. Sequence analysis of IR gene in two patients with adult-onset hyperinsulinemic hypoglycemia and their relatives were performed, and the mutant gene observed in one case was analyzed. Both cases had normal levels of fasting plasma glucose (FPG), fasting hyperinsulinemia, low insulin sensitivity, and hypoglycemia with excessive insulin secretion during oral glucose tolerance test (OGTT). Both reported adult-onset postprandial hypoglycemic symptoms. In one patient, a missense mutation (Arg256Cys) was detected in both alleles of the IR gene, and his parents had the same mutation in only one allele but no hypoglycemia. The other had a novel nonsense mutation (Trp1273X) followed by a mutation (Gln1274Lys) in one allele, and his 9-year old son had the same mutation in one allele, together with hyperinsulinemic hypoglycemia during OGTT. Overexpression experiments of the mutant gene found in Case 1 in mammalian cells showed abnormal processing of the IR protein and demonstrated reduced function of Akt/Erk phosphorylation by insulin in the cells. In two cases of hyperinsulinemic hypoglycemia in adults, we found novel mutations in IR gene considered to be linked to hypoglycemia. We propose a disease entity of adult-onset hyperinsulinemic hypoglycemia syndrome associated with mutations in IR gene.

Key words: Hypoglycemia, Hyperinsulinemia, Insulin receptor mutation

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with type A insulin resistance syndrome, except for a few cases [12, 13], rarely have hypoglycemia.

In this study, we report two cases of adult-onset hyperinsulinemic hypoglycemia, who were found to have a missense mutation in both alleles or a novel nonsense mutation in one allele of the IR gene. The mutations were considered to be linked to hypoglycemia based on cosegregation of the genotype and phenotype in the family relatives and also on functional analysis of the mutant gene in one case.

**Materials and Methods**

**Measurements**

Plasma glucose concentrations were measured by the glucose oxidase method, and insulin and C-peptide levels were measured with enzyme immunoasasy kits. Hemoglobin A1c (HbA1c) of National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) was calculated by the formula HbA1c (%) = 1.02 × HbA1c (Japan Diabetes Society [JDS], %) + 0.25 (%) [15]. Measurement of serum anti-insulin antibodies was outsourced to SRL, Inc. (Tokyo, Japan), and that of serum anti-insulin receptor antibodies to BML, Inc. (Tokyo, Japan) by radioreceptor assay. The latter is based on the detection of antibodies that inhibit $^{125}$I insulin binding in human IM-9 lymphocytes expressing insulin receptors. The insulin/C-peptide molar ratio was calculated by the formula, 1 μU/mL = 6.0 pmol/L on insulin and 1 ng/mL = 0.331 nmol/L on C-peptide.

Three hour 75g OGTT was performed on Case 1 and 5h OGTT was done on Case 2 after overnight fast. Insulin tolerance test (ITT) was performed after overnight fast as described previously [16, 17]. Briefly, blood samples were collected every 3 min for 15 min after intravenous injection of regular insulin (0.1 U/kg), and insulin sensitivity index with the insulin tolerance test (KITT) was calculated from the linear slope of the plasma glucose concentration curve between 3 and 15 min. Fasting test was performed measuring plasma glucose and immunoreactive insulin levels simultaneously for up to 36 h in Case 1 and 48 h in Case 2 to rule out insulinoma.

**Polymerase chain reaction (PCR) direct DNA sequencing**

Genomic DNA was extracted from leukocytes using NucleoSpin Blood XL (Macherey-Nagel, Düren, Germany). Each exon from 1 to 22 of the IR gene was amplified using primers reported previously [18, 19]. Ex-Taq DNA polymerase (Takara Shuzo Co., Biomedical Group, Shiga, Japan) was used to amplify exons 2–22, while AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA) was employed to amplify exon 1. Amplified PCR products were electrophoresed and the size of each product was confirmed. PCR products were sequenced after purification by QIAquick PCR Purification kit (Qiagen, Hilden, Germany). Sequencing reaction was carried out using ABI Prism dye terminator cycle sequencing kit (Applied Biosystems) and the products were electrophoresed and analyzed on an ABI gene analyzer 1100 system according to the protocol supplied by the manufacturer (Applied Biosystems). Genetic analysis in this study was performed with the approval of the Clinical Genetics Unit in our hospital. In all patients, written informed consent was obtained for analysis of their IR gene after genetic counseling had been performed. In genetic counseling, we explained what we can learn from the genetic test, the merits and demerits of knowing the results, and that each patient has the right to decide whether they wish to know the results or not. After the tests, the results were explained to the patients and families if they wished to know what they were.

**Restriction Fragment Length Polymorphism (RFLP) study**

To check whether or not the two mutation sites found in exon 22 in Case 2 (see Results section) were located on the same allele, we performed the following RFLP study. When PCR products of exon 22 were digested with Cac8I, products without mutations ought to be digested to 355, 100, and 55 bps bands and the product with each mutation or both of the mutations should be digested to 410 and 55 bps bands. Thus, the appearance of 355 bps band should denote the presence of a normal allele without mutations, meaning that both mutations should be on the same allele.

**Plasmid and site directed mutagenesis**

The pCMV expression vector used was purchased from Clontech (Mountain View, CA). Expression plasmid for Flag-IR was constructed so that the 8 codons of the Flag epitope were fused to the C terminus of IR using PCR primers. IR$^{R256C}$ DNA was generated by PCR-based mutagenesis and the mutant was verified by direct sequencing. The flag epitope was also fused to IR$^{R256C}$. 
Hyperinsulinemic hypoglycemia with IR mutation

Case reports

Case 1 was a 42-year-old man. He had experienced recurrent episodes of finger tremors and cold sweat, which was dispelled by drinking fruit juice, since he was about 30 years old. During a visit to the general practitioner, he was found to have hypoglycemia (plasma glucose, 50 mg/dL), and was accordingly referred to our hospital for further management. Physical examination and laboratory tests are shown in Table 1. His body mass index (BMI) was 20.7. No signs of acanthosis nigricans or hypertrichosis were noted. Liver function tests, renal function tests, and serum electrolytes were within normal range. Endocrine profiling showed no abnormalities in fasting glucagon, ACTH, cortisol, TSH, or FT3, FT4 levels. Both anti-insulin antibody and anti-insulin receptor antibody were negative. A 75g OGTT showed hyperinsulinemia (Table 2). He developed symptoms of hypoglycemia at 3 h during the OGTT. Fasting insulin/C-peptide ratio was high at 0.25. For the differential diagnosis of hypoglycemia, the patient underwent fasting test and it showed plasma insulin levels decreasing along with glucose levels for up to 36 h (plasma insulin, 1.3 µU/mL; plasma glucose, 53 mg/dl). Furthermore, abdominal dynamic enhancement computed tomography (CT) scan detected no space-occupying lesion in the pancreas, suggesting that insulinoma was unlikely in this case. In addition, he did not have gastrectomy in the past.

Case 2 was a 34-year-old man. Since he was about 15, he experienced recurrent episodes of finger tremors and cold sweat relieved by drinking fruit juice. His body weight was 59.0 kg when he was 20 years old, and it gradually increased up to 70.7 kg over the next 14 years. He consulted the family physician when he experienced repeated recurrence of these symptoms three to four times a week. Blood tests showed hyperinsulinemic hypoglycemia (FPG, 62 mg/dL; fasting immunoreactive insulin (F-IRI), 80.7 µU/mL), for which he was referred to the municipal hospital for further examination. He developed symptoms of hypoglycemia at 3 h during the OGTT. Fasting insulin/C-peptide ratio was high at 0.25. For the differential diagnosis of hypoglycemia, the patient underwent fasting test and it showed plasma insulin levels decreasing along with glucose levels for up to 36 h (plasma insulin, 1.3 µU/mL; plasma glucose, 53 mg/dl). Furthermore, abdominal dynamic enhancement computed tomography (CT) scan detected no space-occupying lesion in the pancreas, suggesting that insulinoma was unlikely in this case. In addition, he did not have gastrectomy in the past.

Table 1 Clinical characteristics of the patients

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Son of case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42</td>
<td>34</td>
<td>9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.8</td>
<td>70.7</td>
<td>20.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170</td>
<td>159</td>
<td>117</td>
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<tr>
<td>Body mass index</td>
<td>20.7</td>
<td>28.0</td>
<td>15.1</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.7</td>
<td>6.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>76</td>
<td>104</td>
<td>89</td>
</tr>
<tr>
<td>Fasting-IRI (µU/mL)</td>
<td>19.0</td>
<td>71.7</td>
<td>14.2</td>
</tr>
<tr>
<td>Fasting-C-peptide (ng/mL)</td>
<td>1.37</td>
<td>3.10</td>
<td>0.90</td>
</tr>
<tr>
<td>IRI/CPR (molar ratio)</td>
<td>0.25</td>
<td>0.42</td>
<td>0.29</td>
</tr>
</tbody>
</table>

IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity
was unlikely. In addition, he did not have gastrectomy in the past.

**Genetic analysis of insulin receptor in the two cases**

To investigate whether insulin receptors in the two patients were normal or not, we conducted sequence analysis of insulin receptor genes. In Case 1, a novel homozygous missense mutation was identified, with Arg (CGC) at 256 replaced with Cys (TGC) (Fig. 1). In addition, two single nucleotide polymorphisms (SNPs) were detected in INSR gene (rs2229429 and rs1799817).

In Case 2, two novel heterozygous mutations were also identified: one was nonsense mutation in which Trp (TGG) at 1273 was replaced with stop codon (TGA), and the other was missense mutation in which Gln (CAA) at 1274 was replaced with Lys (AAA) (Fig. 2a). No other mutations and SNPs were detected in insulin receptor gene in case 2.

Digestion of the normal insulin receptor DNA (Exon 22) with Cac8I yields three bands of 55, 355, and 100 bps. However, digestion of the insulin receptor DNA (Exon 22) from Case 2 yielded two bands: a 410 bps band and a 355 bps band (Fig. 2b), indicating the presence of both the normal allele and mutant allele as well as the presence of two mutations in the same allele.

**Laboratory data and genetic analysis in relatives of the two cases**

Analysis of the family of Case 1 identified impaired glucose tolerance pattern on the 75g OGTT in the test results are shown in Table 1. His body mass index (BMI) was 28.0. No evidence of acanthosis nigricans or hypertrichosis was noted. Other tests showed mild liver dysfunction and hyper LDL-cholesterolemia. Renal function tests and serum electrolytes were within the normal ranges. Endocrine examination showed no abnormalities in fasting levels of glucagon, ACTH, cortisol, GH, TSH, FT3, FT4, or catecholamine. Both anti-insulin antibody and anti-insulin receptor antibody were negative.

A 75g OGTT exhibited diabetic pattern with hyperinsulinemia (Table 2). During the test, he developed symptoms of hypoglycemia (plasma glucose was 46 mg/dL) at 4 h of the OGTT. Fasting insulin level was 41.8 μU/mL, and post-load insulin levels were markedly elevated with a peak concentration of 1050 μU/mL at 2 h. Insulin/C-peptide ratio increased from 0.33 (0 h) to 1.18 (5 h) during OGTT. Insulin tolerance test showed strong insulin resistance (KITT 0.97). For the differential diagnosis of hypoglycemia, fasting test showed plasma insulin decreasing along with glucose levels for up to 48 h (plasma insulin, 227.6 μU/mL to 16.2 μU/mL; plasma glucose, 101 to 60 mg/dL). Abdominal dynamic enhancement computed tomography (CT) showed no space occupying lesions in the pancreas. The sensitivity of CT for the detection of islet-cell tumors varies from 71 to 82 % [20], although the specificity of CT for insulinoma in patients with hyperinsulinemic hypoglycemia is unclear. Together with the result that plasma insulin decreased drastically along with glucose levels, we judged that insulinoma was unlikely. In addition, he did not have gastrectomy in the past.

**Table 2 OGTT data in patients**

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
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<th>90</th>
<th>120</th>
<th>150</th>
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<tbody>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>87</td>
<td>162</td>
<td>201</td>
<td>155</td>
<td>132</td>
<td>71</td>
<td>53</td>
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<tr>
<td>IRI (μU/mL)</td>
<td>32</td>
<td>118</td>
<td>199</td>
<td>320</td>
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<td>329</td>
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<td>Plasma glucose (mg/dL)</td>
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<td>194</td>
<td>243</td>
<td>233</td>
<td>171</td>
<td>46</td>
<td>33</td>
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<tr>
<td>IRI (μU/mL)</td>
<td>41.8</td>
<td>247</td>
<td>429</td>
<td>739</td>
<td>1050</td>
<td>1020</td>
<td>532</td>
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<td>C-peptide (ng/mL)</td>
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<td>IRI/CPR molar ratio</td>
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<td>0.58</td>
<td>0.57</td>
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<td>1.00</td>
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<table>
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<th>Son of case 2</th>
<th>Time (min)</th>
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<th>60</th>
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<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
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<tbody>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>79</td>
<td>164</td>
<td>110</td>
<td>130</td>
<td>119</td>
<td>46</td>
<td>78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRI (μU/mL)</td>
<td>15</td>
<td>189</td>
<td>107</td>
<td>131</td>
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<td>18</td>
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<td>7</td>
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<tr>
<td>C-peptide (ng/mL)</td>
<td>0.9</td>
<td>6.0</td>
<td>4.5</td>
<td>5.1</td>
<td>5.2</td>
<td>1.3</td>
<td>0.4</td>
<td>0.5</td>
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</tr>
<tr>
<td>IRI/CPR molar ratio</td>
<td>0.31</td>
<td>0.57</td>
<td>0.43</td>
<td>0.47</td>
<td>0.50</td>
<td>0.24</td>
<td>0.29</td>
<td>0.25</td>
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OGTT, oral glucose tolerance test; IRI, immunoreactive insulin
Hyperinsulinemic hypoglycemia with IR mutation

Fig. 1  Case 1. Sequence analysis of the insulin receptor.
Nucleotide sequence of the insulin receptor gene in Case 1 is shown in the right panel and compared with that from the control in the left.

Fig. 2  (a) Case 2. Sequence analysis of the insulin receptor
Both Case 2 and his son had similar heterozygous mutations, which were Trp1273X followed by Gln1274Lys.
(b) Restriction fragment length polymorphism (RFLP) study
PCR products of exon22 from Case 2 were digested with \textit{Cac8I}. Two bands were observed: a 410-bps band and a 355-bps band, indicating the presence of both the normal allele and mutant allele and that the two mutations were present in the same allele.
Kuroda et al. with empty vector showed very little or no IR protein, indicating that endogenous IR protein is much less than exogenous one (Fig. 3).

**Insulin induces Akt phosphorylation in cells overexpressing wild type IR and mutant IR\(^{R256C}\)**

Fig. 4 shows Akt-phosphorylation by insulin in HEK293A cells overexpressing wild type IR and mutant IR\(^{R256C}\). Both types of cells showed dose-dependent increase in Akt-phosphorylation after stimulation with insulin. Interestingly, at low insulin con-

**Western blot analysis of the mutant insulin receptor detected in Case 1**

In the next step, pCMV bearing Flag-IR or Flag-IR\(^{R256C}\) was transfected into HEK293A cells, and IR protein was evaluated by western blot using anti-flag and anti-IR antibodies. Western blot analysis of mutant IR showed extremely low levels of mature processed receptor β-subunit, with resultant increase in pro-receptor/mature receptor β-subunit ratio, compared to wild type in experiments of both anti-flag and anti-IR antibody (Fig. 3). Control HEK293A cells transfected with empty vector showed very little or no IR protein, indicating that endogenous IR protein is much less than exogenous one (Fig. 3).

**Table 3 OGTT data of the parents in case 1**

<table>
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<tr>
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<th>Father</th>
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</thead>
<tbody>
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<td>Time (min)</td>
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<td>30</td>
<td>60</td>
<td>90</td>
<td>120</td>
<td>180</td>
<td>240</td>
<td>300</td>
</tr>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>113</td>
<td>210</td>
<td>238</td>
<td>229</td>
<td>195</td>
<td>107</td>
<td>69</td>
<td>88</td>
</tr>
<tr>
<td>IRI (μU/mL)</td>
<td>18.0</td>
<td>93.0</td>
<td>104</td>
<td>142</td>
<td>185</td>
<td>50.0</td>
<td>16.0</td>
<td>8.0</td>
</tr>
<tr>
<td>CPR (ng/mL)</td>
<td>1.93</td>
<td>5.37</td>
<td>6.64</td>
<td>8.17</td>
<td>9.86</td>
<td>5.21</td>
<td>2.64</td>
<td>1.60</td>
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<tr>
<td>IRI/CPR molar ratio</td>
<td>0.16</td>
<td>0.31</td>
<td>0.28</td>
<td>0.32</td>
<td>0.34</td>
<td>0.17</td>
<td>0.11</td>
<td>0.09</td>
</tr>
</tbody>
</table>

|                | Mother |               |               |               |               |               |               |               |
| Time (min)     | 0      | 30            | 60            | 90            | 120           | 180           | 240           | 300           |
| Plasma glucose (mg/dL) | 99     | 109           | 105           | 184           | 204           | 163           | 80            | 83            |
| IRI (μU/mL)    | 10.8   | 51.3          | 15.4          | 62.1          | 84.6          | 47.8          | 5.9           | 6.0           |
| CPR (ng/mL)    | 1.36   | 4.65          | 2.65          | 5.05          | 6.85          | 6.05          | 2.47          | 1.70          |
| IRI/CPR molar ratio | 0.14   | 0.20          | 0.11          | 0.22          | 0.22          | 0.14          | 0.04          | 0.06          |
Hyperinsulinemic hypoglycemia with IR mutation

Since cosegregation of the genotype and phenotype association was observed in all family members, the authors labeled the case as a novel syndrome of autosomal-dominant hyperinsulinemic hypoglycemia linked to a mutation in the IR gene [12]. A few years later, Huang et al. [13] reported a similar familial case associated with heterozygous mutation of Arg1201Trp, adding support to the notion that R1201 of the IR gene is the responsible locus in this syndrome.

Similarly to the above cases, we experienced two independent cases of adult-onset hyperinsulinemic hypoglycemia, who were found to have a missense or a novel nonsense mutation of the IR gene. In Case 2, we identified a novel heterozygous nonsense mutation (Trp1273X) in the IR gene. The mutation could result in products of truncated IR that lack two tyrosine residues (Tyr1355 and Tyr1361), which are phosphorylated following insulin binding to the IR [10]. Since there are some reports that the truncated IR including the two tyrosine residues influence autophosphorylation [23, 24], the truncated IR found in Case 2 might lessen the signal transduction of IR after binding to insulin and concentrations (0.1 and 1.0 nM), Akt phosphorylation was significantly lower in cells overexpressing mutant IR R256C compared to those overexpressing wild-type IR (Fig. 4A). Similar results were observed in insulin-induced Erk phosphorylation in the same cells (Fig. 4B).

**Discussion**

Mutations in IR gene are known to cause insulin resistance and hyperinsulinemia, but they are associated with heterogeneous phenotypes. Leprechaunism and Rabson-Mendenhall syndrome usually have homozygous or compound heterozygous mutations in IR [11], and afflicted patients present with severe insulin resistance and occasional hypoglycemia from infancy. On the other hand, patients with type A insulin resistance syndrome usually have heterozygous mutations in IR [22] with mild insulin resistance, acanthosis nigricans and impaired glucose tolerance, but rarely present with hypoglycemia. Højlund et al. [12] reported a case of familial IR mutation characterized by reactive hypoglycemia, fasting and postprandial hyperinsulinemia, and high serum insulin/C-peptide ratio, in association with heterozygous mutation of Arg1201Gln in the IR gene. Since cosegregation of the genotype and phenotype association was observed in all family members, the authors labeled the case as a novel syndrome of autosomal-dominant hyperinsulinemic hypoglycemia linked to a mutation in the IR gene [12]. A few years later, Huang et al. [13] reported a similar familial case associated with heterozygous mutation of Arg1201Trp, adding support to the notion that R1201 of the IR gene is the responsible locus in this syndrome.

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Similar to the above cases, we experienced two independent cases of adult-onset hyperinsulinemic hypoglycemia, who were found to have a missense or a novel nonsense mutation of the IR gene. In Case 2, we identified a novel heterozygous nonsense mutation (Trp1273X) in the IR gene. The mutation could result in products of truncated IR that lack two tyrosine residues (Tyr1355 and Tyr1361), which are phosphorylated following insulin binding to the IR [10]. Since there are some reports that the truncated IR including the two tyrosine residues influence autophosphorylation [23, 24], the truncated IR found in Case 2 might lessen the signal transduction of IR after binding to insulin and concentrations (0.1 and 1.0 nM), Akt phosphorylation was significantly lower in cells overexpressing mutant IR R256C compared to those overexpressing wild-type IR (Fig. 4A). Similar results were observed in insulin-induced Erk phosphorylation in the same cells (Fig. 4B).
cause insulin resistance.

On the other hand, in Case 1, we identified a homozygous missense mutation (Arg256Cys) located in the cysteine-rich domain of the IR gene. This mutation was one of two compound heterozygous mutations (Cys186Phe and Arg256Cys) which Thiel et al. had already reported in a 13-year-old girl with Rabson-Mendenhall syndrome [25]. The majority of mutations in this domain have been reported to inhibit the cleavage of IR precursor, leading to impaired transport of mature IR to the cell surface [26]. Our overexpression experiments of the mutant gene in HEK293 cells also demonstrated high pro-receptor/mature receptor β-subunit ratio compared to the wild-type, suggesting abnormal processing of the insulin receptor protein. This result is compatible with those reported previously on other mutations in the same domain [27]. This mutation might have reduced stability as well as initiated defective processing into the mature IR. In addition, the R256C substitution introduces an unpaired cysteine residue into the alpha-subunit of the insulin receptor, leading to the potential to create abnormal intra- or inter-subunit disulfide bonds. In fact, our experiments demonstrated reduced Akt phosphorylation by insulin, at least at 0.1-1.0 nM concentrations, in cells that overexpressed the mutant IR, compared with the wild-type IR. These results suggest that this mutation can lower insulin signaling and cause mild insulin resistance. The parents of Case 1 were second cousins and genetic analysis showed heterozygous mutations in the IR gene that were observed in the proband (data not shown). However, neither of his parents had remarkable hyperinsulinemia nor hypoglycemia compared with the proband (Table 3). Thus, this mutation, if it is only in one allele, might not cause any significant alteration in insulin signaling. This is compatible with the report by Thiel et al. [25].

It is not clear how the mutations identified in this report result in hypoglycemia. One possible mechanism would be excessive secretion of insulin after glucose (or meal) loading, with resultant persistently high concentrations of insulin that ultimately suppress hepatic glucose output (even at a low blood glucose level), leading to postprandial hypoglycemia. Indeed, the insulin/C-peptide ratio increased during OGTT in Case 2, suggesting reduced degradation or clearance of endogenous insulin, leading to persistently high levels of insulin even after reaching normal glucose level. Furthermore, in both cases, hyperinsulinemic hypoglycemia appeared in adult. It might be because the insulin resistance and the resultant excessive secretion of insulin were increased by aging.

Case 1 is a typical patient with the mutation in the insulin receptor gene, who showed fasting hyperinsulinemia without obesity. By contrast, case 2 has mild obesity with severe hyperinsulinemia. His body weight was 59.0 kg when he was 20 years old, and it gradually increased up to 70.7 kg over a period of 14 years. Thus the patient might have been overeating as a strategy to prevent hypoglycemia as sometimes seen in cases of insulinoma. In this regard, case 2 had a similar clinical course to insulinoma. Genetic analysis might be required to avoid misdiagnosis in such cases where the fasting insulin levels are drastically high compared with glucose levels as in case 2 (Table 2).

Our study has certain limitations. First, we examined only two relatives of Case 1 and one relative of Case 2 to confirm the genotype and phenotype cosegregation. Since Case 2 had heterozygote mutation, suggesting autosomal dominant inheritance, follow-up and screening of the second generation in this family should provide more information about this mutation. The second limitation relates to our experimental results on Akt phosphorylation. We observed Akt and Erk phosphorylation even at baseline without any insulin treatment. Although the mechanism of phosphorylation in these non-insulin-treated cells remains elusive, one possible explanation would be autonomous activation of certain molecules located upstream of Akt or Erk signal pathway in the cells. Nevertheless, our results indicated that the mutant IR resulted in reduced function of Akt phosphorylation by insulin, at least at low concentrations of 0.1 or 1.0 nM, leading to insulin resistance at these levels of insulin. Another limitation is that the insulin receptor functional analysis of the patient’s cells, such as red blood cells or EB virus-transformed lymphocyte culture, had not been performed in this study. And the last limitation to this study is that deletion encompassing exons cannot be excluded by sequencing alone.

In conclusion, we described novel mutations in the IR gene considered to be linked to hyperinsulinemic hypoglycemia in two independent cases. The phenotypes of the two patients were different from those seen in typical type A insulin resistance syndrome, thus they could be called novel phenotypes related to insulin receptor abnormalities. Together with similar cases reported previously, with mutation in Arg1201 locus in
the IR gene, we propose a disease entity of adult-onset hyperinsulinemic hypoglycemia associated with mutations in the IR gene. We recommend IR gene screening in adult patients with unexplained hyperinsulinemic hypoglycemia.

**Contribution Statement**

Y.K. examined the patients, performed all the experiments including DNA sequencing, analyzed the data, and wrote the manuscript. H.I. analyzed the data, contributed to the discussion, and wrote and edited the manuscript. I.M examined the patients, got the laboratory data, and contributed to the discussion. K.F. and A.F. advised the methods, and contributed to the experimental design and the discussion. R.I., A.I., and I.S. contributed to the discussion and reviewed the manuscript.

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**Disclosure**

All authors declare that there are no conflicts of interest related to this work.

**References**


