Identification of a Novel Mutation in Patients with Type A Insulin Resistance Syndrome

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Abstract
Introduction: Type A insulin resistance syndrome is a rare type of congenital insulin resistance often caused by heterozygous mutations in the insulin receptor gene (INSR). The aim of this study was to explore the clinical and genetic characteristics of three patients with type A insulin resistance syndrome from two Chinese families. Methods: The peripheral blood samples were collected from each family member. Whole-exome sequencing was performed on three patients. Results: Patient #1 was diagnosed with hyperinsulinemia at the age of 11 years and presented with hirsutism, acanthosis nigricans, and polycystic ovaries by 13 years. A heterozygous c.3470A>G mutation in the \textit{INSR} gene was identified in patient #1. Patient #2 was a 13-year-old girl who presented with insulin resistance, polycystic ovary, and hyperandrogenemia. A novel c.3601C>G INSR mutation was identified in patient #2. Co-segregated analysis showed that the c.3601C>G mutation was also found in her father, who had hyperinsulinemia and diabetes mellitus, which was consistent with autosomal dominant inheritance. SIFT and PolyPhen-2 predicted that the c.3470A>G and c.3601C>G mutations in \textit{INSR} had damaging effects. Conclusion: Our study expands the genotypic and phenotypic spectrum of type A insulin resistance syndrome. Awareness of the clinical features coupled with \textit{INSR} gene screening is key to early detection and active intervention.
A Novel Mutation of INSR in Type A Insulin Resistance Syndrome

INSR gene, including type III fibronectin regions FnIII-2b and FnIII-3 and TK domains [2, 3]. Mutations in the INSR gene have been found to cause insulin-resistant syndromes of varying severity, such as Donohue syndrome, Rabson-Mendenhall syndrome (RMS), and type A insulin resistance [4, 5]. The first two syndromes are autosomal recessive, usually present with severe features, such as intrauterine and postnatal growth retardation, dysmorphic features, and impaired glucose homeostasis, and often result in death at an early age. In comparison, type A insulin resistance syndrome is an autosomal dominant disease and is characterized by insulin resistance, acanthosis nigricans, and hyperandrogenism.

Although reports have estimated the prevalence of type A insulin resistance syndrome to be 1/100,000, this disease is rarely reported among Chinese individuals [6]. The phenotype and clinical features of type A insulin resistance syndrome are usually varied, making it challenging to differentiate this disease. This study therefore explored the clinical and genetic characteristics of three patients with type A insulin resistance syndrome from two Chinese families.

Materials and Methods

Ethics Statement

This study recruited three patients with type A insulin resistance syndrome from two Chinese families. This study was approved by the Institutional Ethics Committee of the Third Xiangya Hospital. All participants enrolled herein provided written informed consent. The study was carried out in compliance with the ethical principles stated in the World Medical Association Declaration of Helsinki.

Data Collection

Clinical characteristics and peripheral blood samples of the patients were collected. Insulin resistance was measured using the homeostasis model assessment of insulin resistance (HOMA-IR) and hyperinsulinemic euglycemic clamp. HOMA-IR was calculated as fasting glucose (mmol/L) × fasting insulin (FINS, µU/mL)/22.5.

Hyperinsulinemic Euglycemic Clamp

Hyperinsulinemic euglycemic clamp experiments were carried out in all patients at baseline. Clamp experiments were performed as previously described [7]. After a 12-h overnight fast, catheters were inserted into the antecubital vein of both arms for infusion and in the dorsal vein for blood sample collection. A heated box was used on the blood-taking arm to obtain arterialized venous blood. Insulin (Humulin R, Eli Lilly, USA) was administered intravenously at a rate of 40 mU/kg/min for 150 min. Blood samples were drawn using an intravenous catheter in a heated vein, and glucose concentrations were measured at 5-min intervals. Dextrose 20% was infused at variable rates to maintain a glucose level of 5.0 mmol/L. The time taken to reach euglycemia was calculated from the beginning of the experiment to euglycemia. Glucose disposal rate (GDR, mg/kg/min) was measured during steady-state intervals.

Whole-Exome Sequencing

Genomic DNA was extracted from the peripheral blood of the patients and their family members using standard phenol-chloroform procedures. Whole-exome sequencing was performed on three patients. The isolated DNA was sheared on a Bioreruptor UCD-200 (Diagenode) with a size distribution peak of around 200 bp. Samples were diluted, loaded, and sequenced on the HiSeq2500 platform (Illumina, San Diego, CA). Exome data processing and variant annotation were performed as previously described [8]. Selected variants were analyzed using appropriate variant frequency databases (ESP, dbSNP, 1000 Genomes, ClinVar, and Human Genome Mutation Database (HGMD). We applied filtering criteria that required a variant frequency of less than 1% in 1000 Genomes (ExAC and gnomAD databases). All the variants were interpreted according to the standards of the American College of Medical Genetics (ACMG) and categorized as pathogenic, likely pathogenic, variants of uncertain significance, likely benign, or benign.

Sanger Sequencing

Candidate genes identified by whole-exome sequencing were then confirmed in patients and family members by Sanger sequencing. Primer sequences used for polymerase chain reaction amplification and DNA sequencing of INSR (NM_000208) gene exon 19 were as follows: 5′-GATGGCTCACGGAGACCTG-3′ and 5′-ACGGCTCATTATAGACAACTTCC-3′. The primers of INSR gene exon 20 were as follows: 5′-CTGCCCTTCTTTTCCTTGATG-3′ and 5′-GAACCCCTCTTAGGCTCTG-3′.

In silico Analysis

The effects of variants were predicted using SIFT (http://sift.jcvi.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), and MutationTaster (http://www.mutationtaster.org). The alignment of INSR amino acids across different species was performed using AlignX software (Invitrogen).

To evaluate the structural and/or functional effects of missense mutations, mutation residues were mapped onto the crystal structure of the INSR complex with a peptide substrate (Protein Data Bank code: 1ir3) [9, 10]. Known pathogenic mutations annotated in the UniProt (http://www.uniprot.org/) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) databases were also mapped onto the INSR structural model. Molecular graphics images were then prepared using PyMOL (https://pymol.org/).

Results

Clinical Characteristics

Family 1

Patient #1 (shown in Fig. 1a II-1) was an 11-year-old girl referred to our hospital because of her short stature. She was born small for gestational age (SGA) at 39 weeks of gestation, with a birthweight of 2.25 kg (−3.03 standard
deviation score [SDS]) and a height of 46 cm. At 11 years, she went to the local hospital for her short stature. At the time, she was 134 cm tall (−1.5 SD), weighed 26 kg, and had a body mass index (BMI) of 14.5 kg/m². The peak value of the growth hormone stimulation test was 11.3 ng/mL. Laboratory tests showed normal level of insulin-like growth factor-1 (IGF-1; 410.0 μg/L, reference range [RR]: 88–452 μg/L). A radiological examination of the left hand indicated a bone age of 11.6 years (Tanner-Whitehouse 3, TW3). The patient was diagnosed with growth retardation caused by SGA and was prescribed growth hormone at 3.75 U/day. However, after 1 month of growth hormone treatment, high levels of FINS (203.3 mU/L, RR 2.6–24.9 mU/L) and IGF-1 (542 μg/L) were observed. Despite discontinuing growth hormone treatment, FINS levels remained high (120–200 mU/L). She was then referred to our hospital for assessment of hyperinsulinemia.

The patient had a weight and height of 28 kg and 137.5 cm (−2.08 SD), respectively, and was at a pubertal stage of breast development stage B-3 and pubic hair stage PH-3. Hirsutism and acanthosis nigricans were present over the axillae and neck areas. Hormonal data indicated that she was in the prepubertal stage. Her HbA1c level was elevated (6.1%), with an oral glucose tolerance test (OGTT) revealing impaired glucose tolerance and an insulin releasing test (IRT) indicating hyperinsulinemia (shown in Fig. 2a). The HOMA-IR score of 12.3 and low GDR index of 5.29 mg/kg/min (RR >5.8 mg/kg/min) indicated severe IR. The anti-insulin and anti-INSR antibodies were both negative. She was born from a non-consanguineous marriage, OGTT and IRT performed on her parents were normal, and there were no typical clinical manifestations such as acanthosis nigricans in her parents or hyperandrogenism in her mother.

After metformin treatment (500 mg twice daily) for 3 months, HbA1c improved to 5.9% after 3 months but not insulin resistance (HOMA-IR was 17.7). The patient continued to take this dose for the next 2.5 years. When she was 13.5 years old, hormonal data indicated the onset of puberty and hyperandrogenemia: FSH 3.4 mIU/mL (RR 2.1–11.1 mIU/mL), LH 6.5 mIU/mL (RR 1.8–11.9 IU/mL), estradiol 82.36 pmol/L, and testosterone 151.6 ng/dL (RR 6–82 ng/dL). OGTT and IRT showed impaired glucose tolerance and severe hyperinsulinemia (insulin 152.1 and >1,000 mIU/L at 0 and 120 min) (shown in Fig. 2a). Ovarian ultrasound showed polycystic ovary syndrome. Then she received an increased dose of metformin (500 mg three times a day) for 3 months, but insulin resistance did not improve after 3 months of treatment (HOMA-IR was 15.3), and she switched to piogli-
tazone/metformin hydrochloride therapy (150 mg/500 mg twice daily).

Family 2

Patient #2 (shown in Fig. 1c II-1) was a 15-year-old girl referred to our hospital for hirsutism and amenorrhea. The patient was born SGA at 40 weeks of gestation, with a birthweight of 2.6 kg (−1.82 SDS) and a length of 49 cm. Her first menstruation occurred at the age of 13 years, after which she exhibited amenorrhea. The patient was not obese, with a height of 169 cm, weight of 51 kg, and BMI of 17.8 kg/m². Hirsutism was observed on her lip, as well as...

Fig. 2. Baseline and posttreatment OGTT, IRT, and HOMA-IR in the three patients. a Despite metformin treatment, plasma glucose and insulin resistance worsened with the onset of puberty in patient #1. b Although plasma glucose decreased after metformin treatment in patient #2, no apparent improvement in insulin resistance was observed. c Plasma glucose and FINS decreased significantly after metformin and dapagliflozin treatment in patient #3, indicating a relative improvement in insulin resistance.
as upper and lower limbs. Acanthosis nigricans was observed over the axillae and neck areas. Her pubertal stage was at B-4 and PH-4. OGTT and IRT tests indicated diabetes mellitus and severe hyperinsulinemia (shown in Fig. 2b). Her HbA1c level was elevated (7.5%). The HOMA-IR score of 32.4 and GDR index of 5.06 mg/kg/min indicated severe insulin resistance. The anti-insulin and anti-INSR antibodies were both negative. Basal hormonal assessments showed high total serum testosterone (227.6 ng/dL, RR 6–82 ng/dL), free testosterone (6.66 pg/mL, RR 0.00–4.20 pg/mL), and dehydroepiandrosterone sulfate (108.4 μg/dL, RR 65.10–368.00 μg/dL). Congenital adrenal hyperplasia was excluded given the normal values for 17α-hydroxyprogesterone, cortisol, and ACTH rhythm. Ultrasound revealed polycystic ovary syndrome with a small uterus. Metformin treatment (500 mg twice daily) for 3 months improved HbA1c levels (5.4%). Ethinylestradiol and cyproterone acetate was attempted in patient #2 to reduce the effects of hyperandrogenism. Her testosterone and HOMA-IR levels decreased to 138.2 ng/dL and 25.9, respectively. Her father (shown in Fig. 1c: I-1, patient #3) was a 36-year-old male. The patient was not obese and had a height of 165 cm, weight of 60 kg, and BMI of 22.04 kg/m². No obvious acanthosis nigricans and hirsutism were observed. OGTT, HOMA-IR (shown in Fig. 2c), HbA1c (10.0%), and GDR (5.22 mg/kg/min) indicated diabetes mellitus and severe insulin resistance. Patient #3 received short-term continuous subcutaneous insulin infusion treatment during hospitalization, after which he received metformin (1,000 mg twice daily) and dapagliflozin (10 mg once daily) after discharge. After 3 months, OGTT and IRT tests indicated significantly reduced plasma glucose, with HbA1c dropping to 6.2% compared with 10.0% before treatment. His HOMA-IR score decreased from 46.4 to 7.2 after treatment, indicating a significant improvement in insulin resistance (shown in Fig. 2c).

### Mutations Identified by Whole-Exome Sequencing

**Family 1**

A heterozygous missense mutation, c.3470A>G (p. H1157R), was detected in exon 19 of the INSR gene in patient #1 (shown in Fig. 1b). This mutation was not detected in her parents, indicating that it was a de novo mutation. The p.H1157R mutation was first reported in the Chinese population. Bioinformatics software predicted that it was a pathogenic mutation (shown in Table 1). The amino acid alignment of the INSR gene among different species revealed that the histidine at position 1,157 was conserved across all species examined (shown in Fig. 3a). The ACMG guidelines identified the p.H1157R mutation as pathogenic, given that this mutation had the same amino acid change as a previously established pathogenic variant (PS1), an extremely low frequency (PM2), and was predicted to be damaging and disease-causing by SIFT, PolyPhen-2, and MutationTaster (PP3).

**Family 2**

Patient #2 showed a heterozygous mutation, c.3601C>G (p.R1201G), in exon 20 of the INSR gene (shown in Fig. 1d). Co-segregated analysis showed that this mutation was also found in her father (patient #3), which was consistent with autosomal dominant inheritance. A search of the HGMD revealed that this was a novel mutation. Bioinformatics software predicted it to be a pathogenic missense mutation (Table 1). The amino acid alignment of INSR across different species revealed that the arginine at position 1,201 was conserved across all species examined (shown in Fig. 3b). The p.R1201G mutation was absent in controls (1000 Genomes, ExAC, gnomAD, and CNGB) (PM2) and was predicted to be deleterious by multiple computational software programs (PP3). Another variant c.3601C>T (p.R1201W) was included in the HGMD pro database as a disease-causing variant at the exact same site (PM5). Considering the high specificity of the patient’s phenotype for disease caused by pathogenic

| Gene | Mutation | Zygosity | MutationTaster | Polyphen2 | SIFT | ExAC (all)b | 1000 Genomesb | gnomAD exome (all)c |
|------|----------|----------|----------------|-----------|------|-------------|-----------------|---------------------|
| INSR | p.H1157R | Het | Disease_causing | Benign | Damaging | – | – | 0 |
| INSR | p.R1201G | Het | Disease_causing | Possibly_damaging | Damaging(0) | – | – | – |

*Frequency of variation in total ExAC database. b Frequency of variation in 1000 Genomes database. c Frequency of variation in total gnomAD database.
variants of the \textit{INSR} gene (PP4), the variant was finally classified as likely pathogenic (PM2+PM5+PP3+PP4).

\textbf{Structural Analyses of the Mutations}

The two missense mutations were mapped onto the \textit{INSR} protein’s three-dimensional (3D) structure (shown in Fig. 4), which was determined using X-ray crystallography [9, 10]. 3D model analyses showed that His1157 and Arg1201 played a crucial role in \textit{INSR} function and were closely related to each residue located within the TK domain (shown in Fig. 4a–d). The \textit{INSR} p.H1157R mutation was located just upstream of the catalytic bases of the 1,158–1163 TK domain of the \(\beta\) subunit, and the arginine that replaced histidine eliminated the hydrogen bond interactions between residues 1157H and 1159D, which would have impaired \textit{INSR} autophosphorylation and decreased insulin signaling pathway activity [11]. Moreover, the glycine that replaced arginine eliminated the hydrogen bond interactions between residue 1201R and residues 1236P, 1240L, 1242D, and 1249V.

\textbf{Discussion}

To date, more than 500 \textit{INSR} mutations have been reported, including missense mutations, insertions, deletions, splicing mutations, and compound rearrangement. Studies have shown that 59.3\% of type A insulin resistance syndrome mutations are located in the TK domain of \textit{INSR} [3]. The \textit{INSR} p.H1157R and p.R1201G mutations identified in our study were both located in the TK domain of the \(\beta\) subunit, which was consistent with previous reports [3, 12]. The p.H1157R mutation had been reported in a pair of French sisters who presented with insulin resistance, polycystic ovaries, and hyperandrogenemia [11]. Structural analyses suggested that His1157 was located close to the catalytic bases of the 1158–1163 TK domain, which played an important role in catalyzing the phosphorylation transfer reaction and providing specificity in substrate-binding [11]. In silico structural analyses showed that the Arg-to-Gly amino acid substitutions on residue 1201 would have destroyed the hydrogen bond network and changed the local conformation of the substrate-binding region, resulting in reduced substrate-binding and ultimately impairment of \textit{INSR} signaling, which serves as further evidence that the p.R1201G mutation in \textit{INSR} was located in a biologically significant area. However, further cell or animal experiments are needed to clarify the functional consequence of these \textit{INSR} mutations.

Short stature is not a common clinical characteristic of patients with type A insulin resistance syndrome. In our cases, however, both patients #1 and #2 were diagnosed with SGA, with patient #1 even starting short-term growth hormone treatment. Given that insulin signaling is essential for glucose metabolism during the growth and development of fetuses and infants, birthweight (SDS) could predict insulin signaling function. Takasawa et al. [13]
discovered that individuals who had homozygous or compound heterozygous INSR mutations (Donohue syndrome or RMS) displayed considerably lower mean birthweight (SDS) than those who had heterozygous INSR mutations (type A insulin resistance syndrome). The same study also showed that the extent of growth retardation in the fetus is supposedly directly associated with the severity of insulin signaling impairment.

The severity of the type A insulin resistance syndrome clinical phenotype is heterogeneous in its presentation. The family of patient #2 had typical clinical manifestations, with the patient’s father having been diagnosed with diabetes mellitus, albeit without acanthosis nigricans. Phenotypic differences may be explained by environmental factors and the interaction between gonadal steroids and overactivation of the IGF-1 signal transduction pathway between men and women [14, 15]. Furthermore, Shashaj et al. [16] found that HOMA-IR in young girls increases with age and peaks at the age of 13 years, accompanied by a return to prepubertal levels by the end of puberty. Thus, patient #1 aged 13 years and patient #2 aged 15 years were likely in the stage of increasing HOMA-IR as a physiological change, which might explain the result of the worsening of HOMA-IR in spite of metformin introduction. On the other hand, Musso et al. [17] reported that pancreatic β-cell function in patients with type A insulin resistance syndrome declines gradually over time, which may explain the relative decline in pancreatic β-cell function in patient #3. An interesting phenomenon was that fasting serum insulin decreased after treatment in patient #3, while the 2-h post-load serum insulin increased markedly. The mechanism is unclear. The possible explanation is that the patient’s islet function was restored to a certain extent after the toxic effect of hyperglycemic was relieved, and the insulin reactivity to a glucose load was increased. However, due to the obstacle of insulin action, a large amount of insulin needs to be secreted after the glucose load.

The optimal treatment approach for type A insulin resistance syndrome is not clear. Studies have shown that metformin decreases insulin resistance, lowers androgen levels, and initiates menstrual cyclicity in these patients [18, 19]. Thiazolidinedione has also been attempted in patients with type A insulin resistance syndrome, with
variable reported outcomes [20, 21]. Treatment with recombinant human insulin-like growth factor-1 (rhIGF-1) had been reported to be beneficial for β-cell function and glycemic control in extremely severe insulin resistance, such as RMS and type A insulin resistance syndrome [22–24]. However, the effectiveness of rhIGF-1 therapy seems to decrease with disease progression [25], with high costs limiting its use [26, 27]. New oral hypoglycemics, such as sodium-glucose cotransporter 2 (SGLT2) inhibitors, may be potential therapeutic agents for type A insulin resistance syndrome. Hamaguchi et al. [28] reported a 30-year-old Japanese woman with severe insulin resistance, who was diagnosed with SHORT syndrome (a mutation in PIK3R1). Administration of an SGLT2 inhibitor lowered the proband’s hemoglobin A1c level and allowed a reduction in her insulin dose. Another case of lipodystrophy treated successfully with an SGLT2 inhibitor was reported by Kawana [29]. The SGLT2 inhibitor decreased the patient’s insulin resistance and led to the regression of fatty liver. The SGLT2 inhibitor, dapagliflozin, was also attempted in patient #3, which improved the Hba1c and HOMA-IR significantly. SGLT2 inhibitors enhance urinary glucose excretion, which shifts substrate utilization from carbohydrate to fat, and this mechanism may explain the improvement in both insulin resistance and diabetes [30]. However, additional large-scale and long-term clinical investigations are required to establish the standard therapy for type A insulin resistance syndrome.

Conclusions

In summary, our study detected two INSR mutations (H1157R and R1201G) in two families with type A insulin resistance syndrome, and R1201G is a novel mutation, which expands the clinical and genotypic spectrum of type A insulin resistance syndrome. Awareness of the clinical features coupled with INSR gene screening is key to early detection and active intervention.

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Statement of Ethics

The study was conducted in accordance with the Declaration of Helsinki and the protocol was reviewed and approved by an independent Ethic Committee (The Institutional Review Board of Third Xiangya Hospital, Central South University, China), approval number 21094. All participants enrolled herein provided written informed consent.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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Author Contributions

Study concepts were prepared by Ping Jin. The study was designed by Ping Jin and Liling Zhao. Data acquisition was performed by Liling Zhao, Hongmei Dai, and Qin Zhang. Statistical analysis was done by Liling Zhao and Wenmu Hu. Data analysis and interpretation was done by Liling Zhao and Ping Jin. The manuscript was prepared and edited by Liling Zhao and Ping Jin. All the authors approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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