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Case report

Hereditary Severe Insulin-resistance Syndrome and Acanthosis Nigricans Caused by Novel Mutations in the *INSR* Gene

Chongyang C et al. H-SIRS and AN Caused by Novel Mutations in the *INSR* Gene

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#Equal contribution.

Abstract

Most cases associated with Hereditary Severe Insulin Resistance Syndrome (H-SIRS) are linked to mutations in the insulin receptor (*INSR*) gene. Patients with H-SIRS typically manifest symptoms of hyperinsulinemia, insulin resistance, and diabetes mellitus. Other symptoms include impaired glucose regulation, hyperandrogenism, and the presence of acanthosis nigricans (AN). In this report, we present two cases of H-SIRS in female children exhibiting various symptoms, such as hyperinsulinemia, fasting hypoglycemia, postprandial hyperglycemia, overweight, fatty liver, hyperandrogenism, and varying degrees of AN. One patient also presented with mental retardation. Gene sequencing identified specific mutations in the *INSR* gene for both patients: c.2663A > G (p.Tyr888Cys) and c.38_61del (p.Pro13_Ala20del). These mutations have the potential to disrupt the interaction between INSR and insulin, leading to abnormal insulin signaling, insulin resistance, and various clinical manifestations.

Keywords: Insulin receptor; insulin resistance; hyperinsulinemia; hyperandrogenism; impaired glucose regulation; acanthosis nigricans

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Introduction

Insulin resistance is defined as a decrease in the sensitivity of target organs to the normal physiological effects of insulin. Its characteristics include impaired glucose uptake in muscle and adipose tissue, increased hepatic gluconeogenesis and glycogen breakdown, increased risk of obesity, impaired glucose tolerance, abnormal blood lipids, and endothelial dysfunction(1). Hereditary severe insulin-resistance syndrome (H-SIRS) is an extreme form of insulin resistance, accounting for approximately 0.1% to 0.5% of hospitalized patients with diabetes. H-SIRS caused by mutations in insulin receptor (INSR) can be classified into Donahue syndrome (DS), Rabson–Mendenhall syndrome (RMS), and type A insulin resistance syndrome (A-IR). Additionally, type B insulin resistance is associated with the production of insulin autoantibodies(2,3). The clinical phenotypes of H-SIRS include hyperinsulinemia, abnormalities in glucose homeostasis, dyslipidemia, and acanthosis nigricans (AN). It is also characterized by ovarian dysfunction and excessive androgen levels in women. Most DS or RMS patients have bi-allelic gene variations in the INSRα subunit, resulting in more severe symptoms such as intrauterine and postnatal growth retardation, reduced subcutaneous fat, hirsutism, and characteristic facial changes. A-IR patients have heterozygous variations in the intracellular tyrosine kinase domain of the β subunit(2,3).

Patients with AN typically exhibit pigmentation and excessive keratinization in skin folds, resulting in darkening, roughness, or a velvety texture in localized areas. In certain instances, it may progress to nipple- or wart-like patches. Furthermore, the presence of AN correlates with insulin resistance, metabolic syndrome, and polycystic ovary syndrome (PCOS) in overweight and obese children(4). A single-center study conducted among teenagers in the UK revealed that patients with AN exhibited significantly higher median fasting insulin levels, average fasting blood glucose levels, and median insulin resistance index scores in comparison to the control group (215 pmol/L vs. 126 pmol/L; 4.7 mmol/L vs. 4.5 mmol/L; 6.4 vs. 3.7). AN demonstrates an 81% positive predictive value for insulin resistance,

suggesting its utility as a marker for type 2 diabetes mellitus (T2DM) in teenagers(5). Our study contributes to two cases of adolescent H-SIRS patients with AN resulting from novel *INSR* mutations, thereby expanding the genotype and phenotype spectrum of this disease.

1. Patients and research methods

1.1 Case introduction

Patient 1

Medical history:

Female, 12 years old. The patient consulted with the First Hospital of Lanzhou University due to the discovery of thickening of the skin of the neck, armpits, groin, and popliteal fossa with hyperpigmentation for 8 years. Full-term delivery, birth weight and length unknown, no history of complications during pregnancy, introverted personality since childhood, suspected intellectual disability, and no menstruation. No history of similar diseases in the family.

Physical examination:

This patient's height is 159 cm (75th-90th percentile for same age and gender), weight is 65 kg (over 97th percentile for same age and gender), body mass index (BMI) is 25.71 kg/m^2 (over 97th percentile for same age and gender)(6), skin thickening with hyperpigmentation in the neck, armpits, groin, and popliteal fossa, appearing velvety, no skin tags, normal subcutaneous fat, no excessive body hair (Ferriman-Gallwey Score: 4)(7), no striae, no deformities, Tanner stage 2 breast development, no lactation, normal external female genitalia, no clitoromegaly.

Auxiliary examination:

Oral glucose tolerance and insulin release tests revealed fasting hypoglycemia, postprandial hyperglycemia, and hyperinsulinemia (Table 1). In addition, as shown in Table 2: Chromosomal karyotype: 46, XX; normal liver function, kidney function, blood lipid, and autoantibodies, elevated serum uric acid; elevated testosterone levels, normal follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, prolactin androstenediol, and dehydroepiandrosterone; normal adrenocorticotropic hormone and cortisol rhythm, normal 24-hour urinary free cortisol level. Abdominal ultrasonography suggested a fatty liver, and hand radiography for bone age assessment indicated a bone age (between the ages of 15 and 17 years) greater than the actual age (12 years old).

Patient 2

Medical history:

Female, 10 years old. The patient consulted with the First Hospital of Lanzhou University due to the discovery of thickening of the skin of the neck, armpits, groin, and popliteal fossa with hyperpigmentation for 5 years. Full-term delivery, birth weight 2.8 kg, menstruation occured twice when she was 9 years old, with light red, no dysmenorrhea, no blood clots, lasting about 3-4 days each time, but currently has no menstruation. No history of similar diseases in the family.

Physical examination:

This patient's height is 155 cm (over 97th percentile for same age and gender), weight is 60 kg (over 97th percentile for same age and gender), BMI is 24.97 kg/m^2 (over 97th percentile for same age and gender)(6), thickened and pigmented skin in the neck, armpits, groin, and armpits, nipple-like nodules in the neck and armpits with pigmentation, mostly in joint folds, scattered papules on the back like millet grains, normal skin in between the rash, accompanied by itching, increased body hair (Ferriman-Gallwey Score: 9)(7), no stretch marks, no skin tags, breast development, bilateral Tanner stage 2, no lactation, enlarged female external genitalia, enlarged clitoris (about 2-3cm) (Figure 1).

Auxiliary examination:

Oral glucose tolerance test and insulin release test suggest fasting hypoglycemia, diabetes, and hyperinsulinemia (Table 1). In addition, as shown in Table 2: Chromosomal karyotype: 46, XX; liver function, kidney function, blood lipids, and blood uric acid are normal, glycosylated hemoglobin 7%, histone antibody (1:100) weakly positive, negative for anti-islet cell antibody and anti-glutamic acid decarboxylase antibody; elevated testosterone level, follicle-stimulating hormone, luteinizing hormone, estradiol, progesterone, prolactin, androstanediol, and dehydroepiandrosterone are all normal; adrenocorticotropic hormone and cortisol rhythms are normal, 24-hour urine free cortisol level is normal; growth hormone level, insulin-like growth factor-1 elevated, and insulin-like growth factor binding protein-3 are normal; abdominal ultrasonography suggests fatty liver.

According to the symptoms of hyperinsulinemia, fasting hypoglycemia, postprandial hyperglycemia, overweight, fatty liver, hyperandrogenemia in two patients, as well as various degrees of manifestations of AN, it can be preliminarily diagnosed as insulin resistance syndrome.

1.2 Gene sequencing

1.2.1 Methods

Peripheral venous blood (4 mL) was collected from 2 patients. Genomic DNA was extracted using the Qiagen FlexiGene DNA Kit (Qiagen, Germany), and stored at -20℃ for future use. This study adhered to the ethical principles outlined in the Declaration of Helsinki, and all patients signed written informed consent forms approved by the Ethics Committee of the First Hospital of Lanzhou University (No. LDYYLL-2023-487).

The DNA samples were fragmented using an ultrasonic disruptor, resulting in DNA fragments of 150–300 bp. Adapters were added to both ends of the fragmented genome, followed by PCR library amplification and purification to repair the sticky ends. Subsequently, the post-library amplified DNA underwent hybridization

and amplification with probes (Agilent, SureSelect probe enrichment system). The resulting products were purified and quantified. For the gene testing package related to diabetes and insulin resistance genes, including *INSR*, 6q24 region (*PLAGL1*), 11p15 region (*INS*, *KCNJ11*), 6p22 region (*ZFP57*), *HNF4A*, *GCK*, *HNF1A*, *HNF1B* genes using MLPA large fragment detection (Kangxu Medical Laboratory, Beijing, China), Illumina's NextSeq500-amplified products were utilized for paired-end sequencing. Modified DNA polymerase and dNTPs with four fluorescent labels were added, and the fluorescence signal results were counted to obtain Fastaq-formatted data. The CASAVA (1.8.2) software converted the raw data into recognizable base sequences, followed by alignment, SNP, and DIP analyses to obtain information on mutation sites in the target region. Finally, SIFT (http://sift.jcvi.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), and Mutation Taster (http://www.mutationtaster.org) were employed for protein functional damage analysis to qualitatively predict the probability of the results. This process helped identify mutation sites requiring further validation. Gene sequences for the identified mutation sites were retrieved from the GenBank human genome database, and primers were designed and synthesized using the Primer Z website (http://genepipe.ncgm.sinica.edu.tw/primerz/primerz4.do). PCR amplification was performed on the mutation sites, and the obtained sequences were aligned with previous sequences to exclude false-positive sites in second-generation sequencing.

1.2.2 Gene sequencing results

Patient 1, harboring a mutation in exon 13 of the *INSR* gene, was identified with the c.2663A>G mutation. This represents a heterozygous missense mutation where the A nucleotide at position 2663 is substituted with a G nucleotide, resulting in the amino acid at position 888 of the β-subunit of the INSR being converted from a tyrosine to a cysteine (p.Tyr888Cys) (Figure 2). Protein functional analysis using SIFT, PolyPhen, and MutationTaster indicates that the p.Tyr888Cys mutation is anticipated to be "pathogenic" by SIFT (score: 0, disease prediction: Deleterious), PolyPhen2_HVAR software (score: 1, disease prediction: Deleterious), and MutationTaster (score: 1, disease prediction: Deleterious).

Patient 2 manifested an in-frame mutation in exon 1 of the *INSR* gene, resulting in a 24-base pair deletion between the 38th and 61st bases (c.38_61del). This deletion leads to the fusion of the original 37th base C with the 62nd base T, causing the loss of 8 amino acids: Pro13 (P13), Leu14 (L14), Leu15 (L15), Val16 (V16), Ala17 (A17), Val18 (V18), Ala19 (A19), and Ala20 (A20) (p.Pro13_Ala20del). Notably, the translation of the amino acids preceding and following the deletion, Ala12 (A12) and Leu21 (L21), respectively, remained unaltered (Figure 3).

1.3 Treatment and follow-up

Two patients received metformin at a dosage of 500 mg twice daily to address insulin resistance and facilitate weight control. Subsequently, blood sugar levels were effectively managed and maintained within the normal range. Additionally, a noticeable reduction in skin pigmentation was observed (Table 3).

2. **Discussion**

Here, we present findings from two cases involving female pediatric patients who exhibited hyperinsulinemia, fasting hypoglycemia, and postprandial hyperglycemia during glucose tolerance and insulin release tests. Both patients displayed characteristic symptoms of AN, such as skin thickening and pigmentation in the neck, armpits, groin, and other skin folds. Additionally, they presented with hyperandrogenism, overweight, and fatty liver. Notably, our genetic sequencing revealed specific mutations in the *INSR* gene for each patient.

In patient 1, a heterozygous missense mutation, c.2663A>G, in exon 13 of INSR was identified, resulting in the amino acid at position 888 of the β-subunit of the INSR being converted from a tyrosine to a cysteine (p.Tyr888Cys) (Figure 2). Patient 2, in contrast, exhibited a deletion of 24 base pairs between positions 38 and 61 in exon 1 of INSR, leading to a total of 8 amino acid deletions between proline at position 13 and alanine at position 20 in the α-subunit of INSR (p.Pro13_Ala20del) (Figure 3). Consequently, considering the clinical phenotypes and laboratory results of both cases, we diagnosed these patients with H-SIRS. Most mutations were associated with the *H-SIRS* and *INSR* genes. Ardon et al.(8) summarized 132 pathogenic variants of *INSR* mutations, including missense, nonsense, insertion, deletion, and complex rearrangements. Recently, new mutation sites have been identified. You et al.(9) recently reported a patient with hyperinsulinemia associated with AN, and gene sequencing revealed a novel variant, c.3472C>T (p.Arg1158Trp), in the index case and his father's *INSR* gene. Poon et al.(10) reported a case of hyperinsulinism and hypoglycemia in an infant who did not respond to diazoxide treatment. It was later discovered that she carried a heterozygous *INSR* gene mutation, c.1246C>T, leading to the replacement of the arginine codon at position 416 with a stop codon.

Different types of *INSR* gene mutations can affect the molecular structure of the INSR, leading to various consequences. Zhou et al.(11) reported two cases of A-IR and one case of DS: the proband with A-IR and his sister had compound heterozygous mutations c.3670G>A and c.3614C>T in the INSR gene, while the patient with DS had mutations c.749_751del and c.3355C>T. The impact of these new variants on INSR function was determined by expressing the mutant receptors in Chinese hamster ovary (CHO) cells. The results showed that Thr250 and Val1224 are located in the cysteine-rich region and tyrosine kinase domain of INSR, respectively. The new variant c.749_751del (p.Thr250del) in the α subunit reduced the expression of mature INSR protein and severely impaired INSR function. In contrast, although protein function analysis suggested that the c.3670G>A (p.Val1224Met) mutation was pathogenic, the new variant in the β subunit did not affect the expression and phosphorylation of INSR. The tyrosine kinase activity of INSR is crucial for insulin action in vivo, with the α subunit containing the insulin binding site and the β subunit containing the tyrosine kinase domain. Phosphorylation of the INSR β subunit is necessary for mediating insulin action. The

translation products of *INSR* mutations lacking kinase activity do not mediate the promotion of glycogen synthesis, glucose uptake, cell proliferation, or gene transcription by insulin(12).

The two *INSR* gene mutations we report have been present in previous studies, therefore, the molecular structure and function resulting from these two mutations are unknown. However, mutant sites at adjacent positions in the *INSR* gene have been reported previously. Qin et al. (13) reported a case of c.62T > G (p.L21R) and c.2563G > T (p.V855F) mutations in the *INSR* gene, in which the patient presented with thickening of the skin of the neck and trunk accompanied by hyperpigmentation, roughness of the face, enlargement of the head, thickness of the lips, generalized coats of body hair, reduction of subcutaneous fat, and a severe speech disorder. Molecular dynamics simulations showed that the c.62T > G missense mutation located in the α-subunit led to functional defects in the signal peptide, and the c.2563G > T missense mutation was located in the cysteine-rich structural domain of the β-subunit, which completely altered the conformation of the INSR, led to inactivation of the INSR, and interfered with INSR binding to the ligand. In addition, Brierley et al. (14) evaluated the impact of *INSR* gene mutations using a cell culture model. The results indicate that when the *INSR* mutation site is located on the cell surface, the binding of INSR to insulin and signal transduction are impaired. When the aspartic acid placement at position 707 on the β-subunit of the INSR is replaced by an alanine, this mutation is located near the cysteine residue, which may affect disulfide bond formation as well as the autophosphorylation of the INSR and its binding to substrates. Accordingly, the two *INSR* gene mutations identified in this study, with the c.2663A>G (p.Tyr888Cys) mutation site located at the junction between the α and β subunits, may affect transmembrane signaling of insulin. In contrast, the c.38_61del (p.Pro13_Ala20del) mutation site located in the α subunit may affect insulin binding to the receptor, but the specific mechanism still needs further basic research confirmation.

Gene mutations severely impaired the sensitivity of the INSR to insulin, leading to hyperinsulinemia and reduced affinity for peripheral tissue insulin receptors, further promoting insulin resistance. This required pancreatic beta cells to secrete more insulin compensatively, resulting in a vicious cycle. Over time, pancreatic cell function eventually declined, increasing the risk of chronic complications, such as diabetes[3]. The main histological features of AN included hyperkeratosis and epidermoid cyst disease, along with mild or absent acanthosis and excessive basal pigmentation(15). Insulin promoted cell proliferation, and hyperinsulinemia led to elevated circulating levels of insulin-like growth factor 1 (IGF-1), causing overactivation of IGF-1 receptors on fibroblasts and keratinocytes and driving excessive cell proliferation and differentiation. Therefore, AN could be considered a cutaneous manifestation of insulin resistance(16). In addition, both patients had hyperandrogenemia. The cause might be the cross-reactivity between high concentrations of insulin and IGF-1 receptors in the ovaries, leading to excessive secretion of androgens(17).

The treatment of AN depends on the underlying conditions. In cases of insulin resistance, weight control or weight loss surgery can improve symptoms. In addition, the indications for metformin in T2DM have been extended to include PCOS and AN. Limited data are available regarding cosmetic interventions such as melatonin, urea cream, vitamin D analogs, or topical tretinoin. In the early stages, lifestyle changes and improvement of insulin resistance should be started, along with the use of keratolytic agents (such as α hydroxy acids and salicylic acid) in combination with depigmenting agents (such as hydroquinone or azelaic acid). Topical tretinoin can be administered when velvety skin changes are observed(15).

Conclusion

Our study reports two cases of H-SIRS in female children, both presenting with hyperinsulinemia, fasting hypoglycemia, postprandial hyperglycemia, fatty liver disease, hyperandrogenism, and varying degrees of hirsutism. Patient 1 exhibited suspected intellectual disability. Genetic testing unveiled the presence of a c.2663A>G (p.Tyr888Cys) missense mutation and a c.38_61del (p.Pro13_Ala20del) frameshift mutation in *INSR* in both patients. These mutations have the potential to impact the binding of INSR to its ligand, insulin, thereby disrupting insulin receptor binding and resulting in abnormal insulin signaling. This disruption leads to insulin resistance and other associated clinical manifestations. Hyperinsulinemia and insulin resistance are relatively common in clinical practice, but their causes are diverse, therefore, genetic testing is important in determining the etiology of insulin resistance.

There are limitations to this study. The present study failed to further investigate the effect of the mutant site on the molecular structure and function of the INSR, which can be further investigated by in *vivo* or in *vitro* experiments in the future.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (No.LDYYLL-2023-487). Each participants provided written informed consent.

Consent for publication

All the authors agreed to publish this article.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

Chen Chongyang, Zhao Yangting, Li Kai, Lv Xiaoyu, Wang Yawen, Zhen Donghu, Fu Songbo, Ma Lihua, Zhou Liyuan and Liu Jingfang declare that they have no conflict of interest.

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Author contribution

Chen Chongyang, Zhao Yangting and Liu Jingfang conceived and designed the study. Chen Chongyang, Zhao Yangting, Li Kai, Lv Xiaoyu, Wang Yawen, Zhen Donghu, Fu Songbo, Ma Lihua, Zhou Liyuan and Liu Jingfang managed the patients and collected patients' serum for genetic sequencing and bioinformatics analysis. Chen Chongyang, Zhao Yangting and Liu Jingfang contributed to the results interpretation, drafting and revising the paper. All authors read and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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Table 1. Oral glucose tolerance test and islet function determination of the patients.

Glucose levels and Pancreatic island function	Patient 1	Patient 2	Reference ranges
Glu(0min)(mmol/L)	4.46	3.98	3.9-6.1
Glu(30min)(mmol/L)	6.53	10.69	
Glu(60min)(mmol/L)	7.63	11.12	
Glu(120min)(mmol/L)	8.39	11.10	
Glu(180min)(mmol/L)	6.97	11.23	
Ins(0min)(mIU/L)	84.90	95.03	1.9-23
Ins(30min)(mIU/L)	>300.00	>300.00	
Ins(60min)(mIU/L)	>300.00	>300.00	
Ins(120min)(mIU/L)	>300.00	>300.00	
Ins(180min)(mIU/L)	>300.00	>300.00	

Note: Glu: Glucose; Ins: Insulin.

Table 2.Summary table of the clinical data of the patients

Inspection item		Patient 1	Patient 2	Reference ranges
Gender		Female	Female	
Age(year)		12	10	
chromosome		46, XX	46, XX	
BMI(kg/m^2)		25.71	24.97	Overweight: greater than or equal to the 85th percentile of BMI specific to age and gender (10 years female BMI≥19.60; 12 years female BMI≥21.12). Obesity: greater than or equal to 90th percentile for age and sex specific BMI (10 years female BMI≥22.60; 12 years female BMI≥24.89).(7)
Laboratory investigations	AST(U/L)	25	14	14-44
	ALT(U/L)	28	16	7-30
	Scr(μmol/L)	52.1	47.90	27-66
	SUA(μmol/L)	103	337	125-420
	TC(mmol/L)	2.63	3.74	3.6-5.7
	TG(mmol/L)	1.42	1.18	0.8-1.8
	LDL-c(mmol/L)	1.62	2.46	1.55-3.7
	HDL-c(mmol/L)	0.79	0.86	0.8-1.8
Abdominal ultrasound		Fatty Liver	Fatty Liver	
GH(ng/mL)			0.36	0.123-8.050
IGF-1(ng/mL)			543.00	123.0-427.0
IGFBP-3(ng/mL)			5310	3116-6761
Autoantibodies	ANA	negative	negative	negative
	AMA	negative	negative	negative
	ANuA	negative	negative	negative
	AHA	negative	weakly positive	negative
Sex hormones	FSH(mIU/mL)	5.41	3.47	follicullar phase: 3.50-12.50; ovulatory period: 4.70-21.50; luteal phase: 1.70-7.70; menopause: 25.80-134.80
	LH(mIU/mL)	8.04	9.05	follicullar phase: 2.40-12.60; ovulatory period: 14.00-95.60; luteal phase: 1.00-11.40; menopause: 7.70-58.50
	E2(pg/mL)	24.50	25.20	follicullar phase: 12.40-233.00; ovulatory period: 41.00-398.00; luteal phase: 22.30-341.00; menopause: 0-138.00; early pregnancy: 154.00-3243.00; middle pregnancy: 1561.00-21280.00; late pregnancy: 8525.00-30000.00
	PROG(ng/mL)	0.200	0.420	follicullar phase: 0.057-0.893; ovulatory period: 0.121-12.00; luteal phase: 1.83-23.90;

				menopause: 0-0.126; early pregnancy: 11.00-44.30; middle pregnancy: 25.40-83.30; late pregnancy: 58.70-214.00
	PRL(ng/mL)	7.49	16.80	4.79-23.30
	T(ng/dL)	41.70	70.90	Tanner by stages: 1: 0-6.10 2: 0-10.40 3: 0-23.70 4: 0-26.80 5: 4.60-38.30
	DA(ng/mL)	1.67	3.18	0.3-3.5
	DHEA(μg/dL)	64.60	79.20	35-430
	ACTH(8 A. m)(pg/mL)	47.80	39.50	7.20-63.30
	ACTH(4 P. m)(pg/mL)	19.10	38.80	
	ACTH(0 A. m)(pg/mL)	8.85	10.60	
Cortisol and ACTH rhythm	Cor(8 A. m)(μg/dL)	11.70	7.25	6.02-18.40
	Cor(4 P. m)(μg/dL)	4.40	6.89	2.68-10.50
	Cor(0 A. m)(μg/dL)	1.07	0.74	
	24hUHC(μg/24h)	365.52	264.84	75.0-520.0

Note: BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Scr: Serum creatinine; SUA: serum uric acid; TC: Total cholesterol; TG: Triglycerides; LDL-c: Low-density lipoprotein cholesterol; HDL-c: High-density lipoprotein cholesterol; GH: Growth hormone; IGF-1: Insulin-like growth factor 1; IGFBP-3: Insulin-like growth factor binding protein 3; ANA:Antinuclear antibody; AMA:Anti-mitochondrial antibody; ANUA: Anti-nucleosome antibody; AHA: Anti-histone antibody; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol 2; PROG: Progesterone; PRL: Prolactin; T: Testosterone; AD: Androstendione; DHEA: Dehydroisoandrosterone; ACTH:Adrenocorticotropic Hormone; Cor:Cortisol; UFC:urinary-free cortisol.

Table 3.Results of follow-up examinations

Glucose levels and Pancreatic island function	Patient 1	Patient 2	Reference ranges
Glu(0min)(mmol/L)	3.82	3.77	3.9-6.1
Glu(120min)(mmol/L)	-	4.48	
Ins(0min)(mIU/L)	37.00	54.68	1.9-23
Ins(120min)(mIU/L)	-	>300.00	
T(ng/dL)	54.00	40.40	Tanner by stages: 1: 0-6.10 2: 0-10.40 3: 0-23.70 4: 0-26.80 5: 4.60-38.30

Note: Glu: Glucose; Ins: Insulin; T: Testosterone.

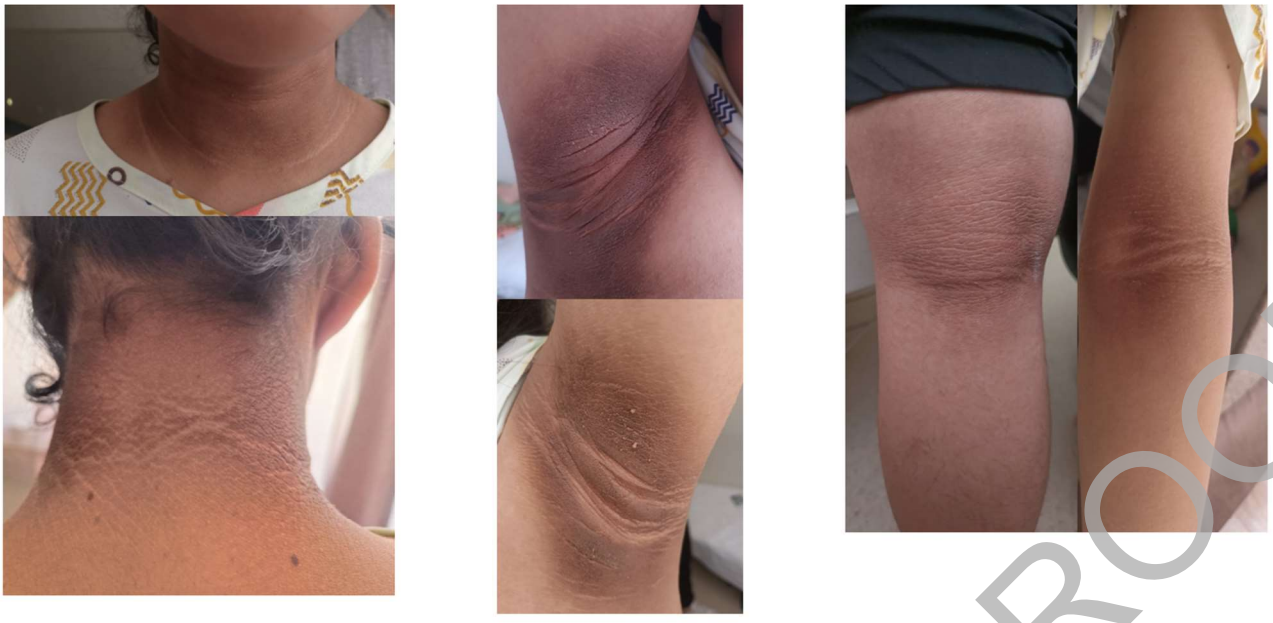
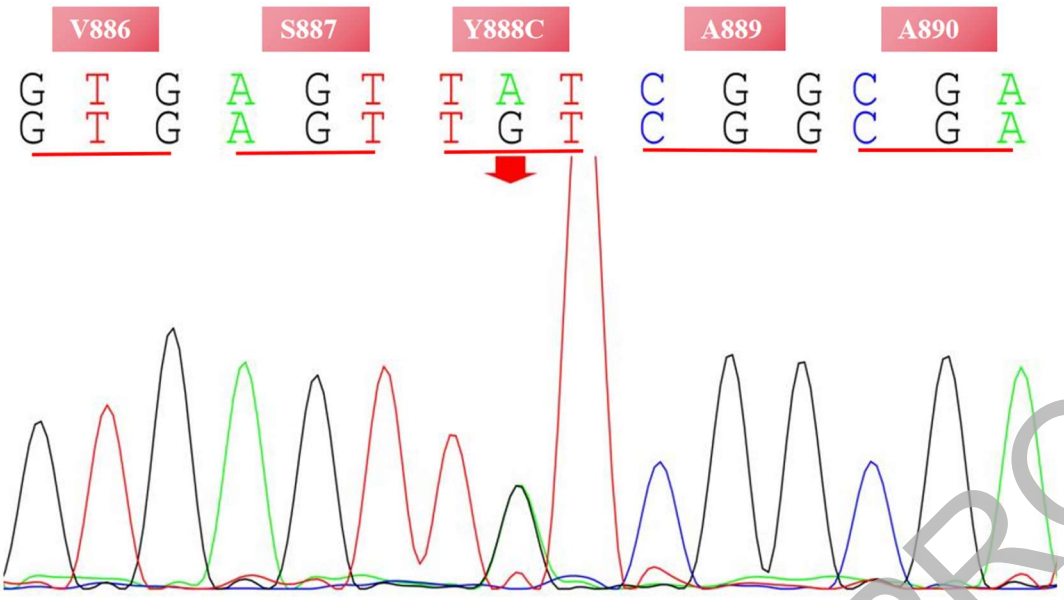
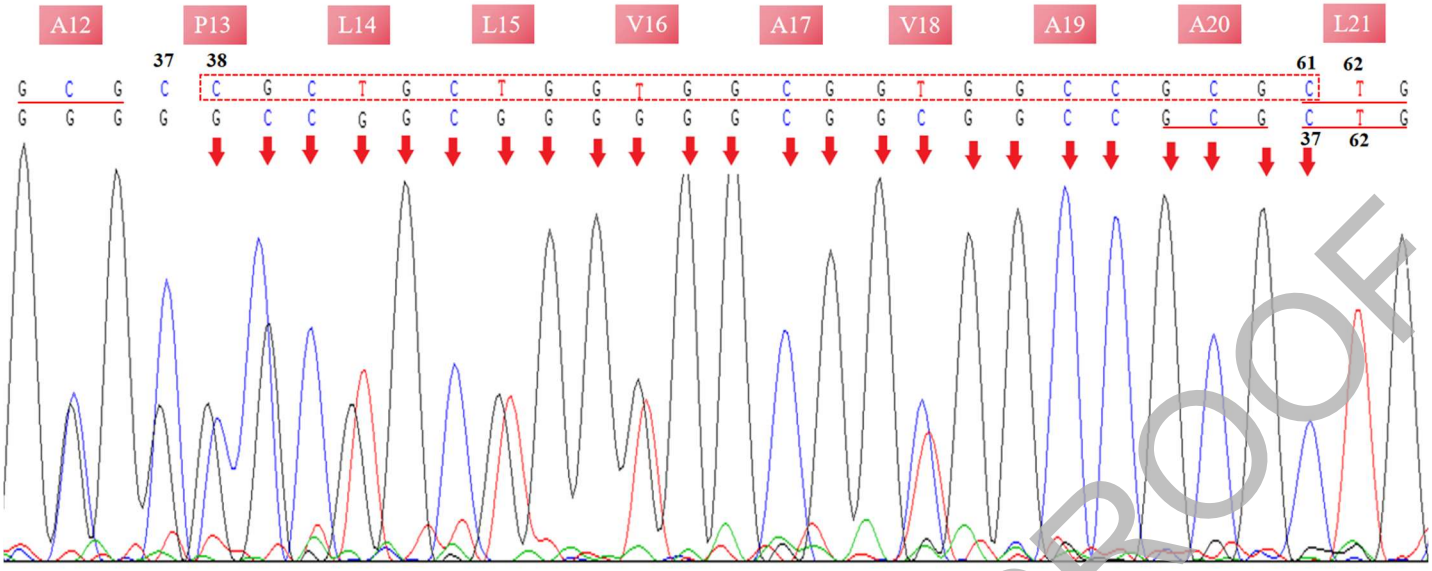


Figure 1. Pedigree of Patient 2 and photos of pioneer. Patient 2 has thickening of the skin with pigmentation in the neck, axilla, groin, and axilla, and nipple-like nodules can be seen in the neck and axilla.



Gene sequencing in Patient 1. *INSR* Exon 13: c.2663A>G(p.Tyr888Cys)

Figure 2. Gene sequencing in Patient 1. *INSR (NM_000208)*: Chromosome location: chr19:7141707, Exon13; Nucleotide change: c.2663A>G; Amino acid change: p.Tyr888Cys; Mutation type: missense mutation; Validation result: heterozygous.



Gene sequencing in Patient 2. *INSR* Exon 1: c.38_61del(p.Pro13_Ala20del)
Note: The dashed line represents a missing base pair; the solid line represents the same amino acid.

Figure 3. Gene sequencing in Patient 2 and her parents. *INSR (NM_000208)*: Chromosome location: chr19:7293842, Exon1, nucleotide change: c.38_61del; amino acid change: p.Pro13_Ala20del; mutation type: in-frame mutation, verification result: heterozygous.

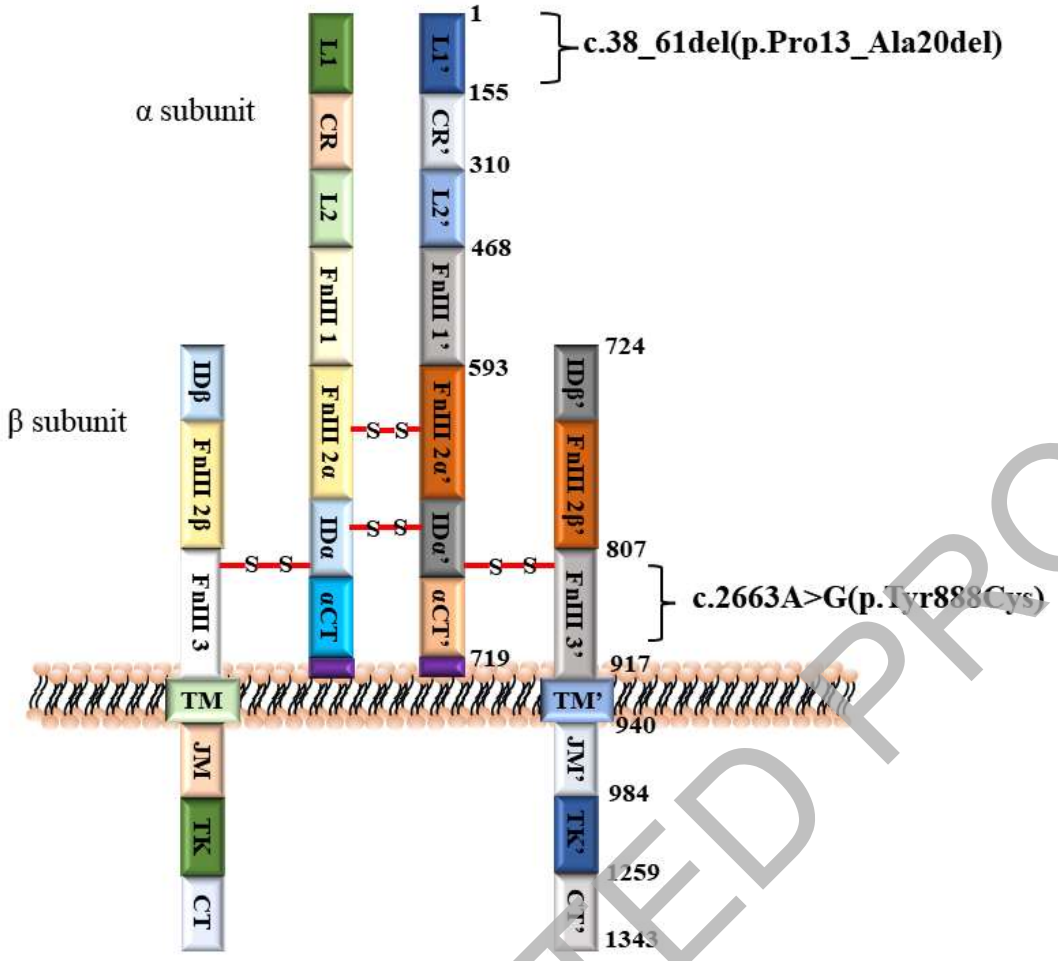


Figure 4. Insulin Receptor Structural Model and Mutation Site Localization. The INSR is composed of two α subunits and two β subunits connected by disulfide bonds (-s-s-). The INSRα subunit acts as the ligand-binding site and consists of a leucine-rich repeat-1 (L1), a cysteine-rich region (CR), a leucine-rich repeat-2 (L2), two fibronectin type III domains (FnIII-1 and FnIII-2α), an insert domain α (IDα), and an α-helical C-terminal domain (αCT). The INSRβ subunit includes an extracellular insert domain β (IDβ), fibronectin type III domains (FnIII-2β and FnIII-3), a transmembrane helix (TM), an intracellular juxtamembrane region (JM), a tyrosine kinase domain (TK), and a C-terminal tail (CT). Based on the genetic sequencing results of two patients reported in our study, the c.2663A>G mutation may affect the transmembrane signaling of insulin, and the c.38_61del mutation may affect the binding of insulin to the receptor.