Case Report

**Title:** One novel 2.43Kb deletion and one single nucleotide mutation of the \(\text{INSR}\) gene in a Chinese neonate with Rabson-Mendenhall syndrome

**Short Running Title:** A novel 2.43Kb deletion in \(\text{INSR}\) gene

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**What is already known on this topic:**
Mutation of \(\text{INSR}\) is responsible for Donohue syndrome (DS) and Rabson-Mendenhall syndrome (RMS). These two diseases are both characterized as insulin resistance. Typical symptoms of RMS include growth retardation, elfin face, gingival hyperplasia, acanthosis nigricans, hypertrichosis and insulin resistance.

**What this study adds:**
We report an atypical and mild RMS patient with novel 2.43Kb deletion and a known-pathogenic point mutation in \(\text{INSR}\) gene as compound heterozygous.

**Abstract**
Mutations in insulin receptor gene (\(\text{INSR}\)) are responsible for Donohue syndrome (DS) and Rabson-Mendenhall syndrome (RMS). These two diseases are both characterized as insulin resistance. A Chinese neonate suffering from glucose homeostasis, hyperinsulinemia, dry skin, heavy hair, elevated testosterone and growth retardation was recruited. To search for candidate point mutations, small insertions or deletions and copy number variants, 2742 inherited disease-gene panel sequencing was performed. One pathogenic mutation c.3355C>T, p.Arg1119Trp and a novel 2.43Kb deletion (chr19:7150507-7152938) in \(\text{INSR}\) were found in this patient. The patient was diagnosed as RMS. Sanger sequencing and real-time quantitative PCR confirmed the missense variant and microdeletion respectively. We
therefore supposed that these variants were candidate mutations of this family. We report a novel 2.43Kb deletion in INSR gene and provide further proof to the power of NGS in rare disease diagnosis.

**Keywords:** Insulin receptor gene (*INSR*); Rabson-Mendenhall syndrome (RMS); Neonate; Mutation; Next generation sequencing (NGS)

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**Introduction**

*INSR* is the disease-causing gene of a series of insulin resistance diseases, including hyperinsulinemic hypoglycemia, familial, 5 (MIM#609968), Donohue syndrome (DS, also called leprechaunism) (MIM#246200) and Rabson-Mendenhall syndrome (RMS) (MIM#262190). The inheritance pattern of DS and RMS is autosomal recessive. Typical symptoms of DS include growth retardation, elfin face, gingival hyperplasia, acanthosis nigricans, hypertrichosis and insulin resistance (1). RMS and DS share similar symptoms. The symptoms of DS are more severe, have an infantile onset and may lead to early death. RMS is often childhood-onset and with survival up to adulthood with milder symptoms. The differential diagnosis is based on the onset age and severity of the disease (2).

In this study, we describe a Chinese male newborn with hyperinsulinemia and hyperglycemia. NGS found compound heterozygous mutations of *INSR*, including one known mutation and one novel 2.43Kb deletion.

**Case reports**

The proband is the first child of a couple of non-consanguineous parent. He was delivered by natural labor at 36 weeks of gestation. During the fetal period, he was diagnosed with intrauterine growth retardation, and oligohydramnios and was born with a low birth weight at 1.7 kg. At 13 days old, he presented heavy hair over whole body and dry skin. The plantar grasp reflex, moro reflex and sucking reflex were weak. Misalignment of teeth, malformation of face, abnormality of mouth size, acanthosis nigricans and abdominal distention were not observed. Clinical test showed hyperglycemia (14.7 mmol/L), hyperinsulinemia (> 300 IU/ml) and fasting hypoglycemia. C-peptide was 4.05 ng/ml (1.10-4.40 ng/ml). HbA1c (glycosylated hemoglobin) was normal (5.4 %). Other abnormal laboratory test results are showed in Table 1. Insulin auto-antibodies were negative. Routine blood test, liver function test, and thyroid-stimulating hormone were normal.

Echocardiography suggested a ventricular septal defect, atrial septal defect and ultrasonography indicated swelling of both kidneys. Magnetic resonance imaging (MRI) of the brain indicated an impaired myelination of white matter. The mother had transient hypothyroidism during pregnancy. The family history is negative. At the last outpatient follow-up, he (4 months old) had a neurodevelopmental delay and still had high postprandial blood glucose (> 11 mmol/L) and hyperinsulinemia (> 300 IU/ml).

Pre-test counseling was performed by physicians and appropriate informed consent was
signed by the patient’s parents in the clinic. The criteria of genetic testing received approval from the ethics committees of Children's Hospital, Fudan University (2016-235). Genomic DNA samples were extracted from whole blood using the QIAamp DNA Blood Mini kit (QIAGEN, Germany) following the manufacturer’s protocol. The quality and quantity of the DNA samples were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Nucleic acid preparation and high-throughput sequencing were performed using standard protocols in a Clinical Laboratory Improvement Amendments (CLIA) compliant sequencing laboratory in Wuxi NEXTCODE (288 Fute Zhong Road, Waigaoqiao Free Trade Zone Shanghai 200131, China CLIA ID 99D2064856). Inherited-Disease panel sequencing is generated using the Agilent ClearSeq Inherited Disease kit, Illumina Cluster and SBS kit. The sequencing is performed using NGS on the Illumina Hiseq 2000/2500 platform. It covers a minimum of 98% of the genome with 20X coverage and is compared to a human reference sequence.

We identified a known-pathogenic mutation (c.3355C>T, p.Arg1119Trp) at exon 18 of the INSR gene (NM_000208). This mutation has been reported in a patient with DS (3). This mutation was recorded in HGMD (http://www.hgmd.cf.ac.uk/ac/index.php) as CM1119. The Exac database (http://exac.broadinstitute.org/) and the 1000 gene database (http://www.internationalgenome.org/) have no record about this variant. This mutation is also the only record in our internal database, which contains sequencing data of ClearSeq of 4071 patients. Paired primers were designed by using Primer3 website and primer-BLAST (5'-GGGAGGAGAACCCTGGTGAG -3' and 5'-ATCCGAGGAGGCCAGGAG-3'). Sanger sequencing indicated that this mutation was inherited from the mother (Fig. 1A, 1B, 1C).

We used CANOES (CNVs with an Arbitrary Number of Exome Samples) for basic detection of CNVs from NGS data at gene-level and region-level (4). Gene-level annotation was based on Online Mendelian Inheritance in Man (OMIM), Human gene mutation database (HGMD), Swiss-Prot and RefSeq. Region-level information was annotated by DGV (Database of genomic variants), and DECIPHER (Database of genomic variation and Phenotype in Humans using Ensembl Resources). We detected a novel deletion of approximately 2.43Kb (chr19:7150507-7152938) within the INSR gene (Fig. 1D). This deletion is not found in HGMD, DECIPHER or DGV. Additionally, it is absent from our internal database. The detected mutation was confirmed using real-time quantitative PCR. PCR-amplified DNA products were subjected to direct automated sequencing (ABI step one plus v.2.0). Both strands of each amplicon were sequenced using the primers 5'-CCTGACCTGGGGACGAAAA-3' and 5'-GTCTCCACCATTCGAGTCTGA-3'. Real-time quantitative PCR indicated the deletion was from the father (Fig. 1E). This region covers part of exon 10 and all of exon 11. The deletion is estimated to cause a truncated protein. We performed three-dimensional (3D) structural modeling of the monomer form of the insulin receptor and mapped the deletion on to it. The PBD number of the insulin receptor extracellular region is 4ZXB.E, that of the juxtamembrane region is 2MFR.A and that of the tyrosine kinase domain is 3BU3.A (5). The 3D structural modeling shows the monomer form of the insulin receptor (IR). The Fibronectin type-III 2-domain, Fibronectin type-III 3-domain, Insulin in-binding-region, protein kinase-like domain, juxtamembrane region and partial Fibronectin type-III 1-domain are absent (Fig. 2) (5).

Discussion
In this case, we identified a novel microdeletion and a known missense mutation within the \textit{INSR} gene, which caused compound heterozygous mutation in a Chinese newborn baby. The \textit{INSR} gene is located at chromosome 19 and encodes IR. HGMD collected 178 mutations of \textit{INSR}. For RMS, 26 mutations of \textit{INSR} are in records. Of them, two compound heterozygous mutations, each of which contains one deletion and one single nucleotide mutation. A gross deletion containing exons 9 and 10 was reported in a 15-year-old RMS patient(6). This patient carries a mutation (p.Ser635Leu) in \textit{INSR} with a compound heterozygous genotype. The main phenotypes are hyperglycemia and hyperinsulinemia. Another gross deletion contains exon 18. This RMS patient, as a compound heterozygous, also carried a mutation (p.Val66Ala). Nephrocalcinosis is one of the patient’s dominant features (7). For the patient we report, the missense mutation (c.3355C>T, p.R1119W) has been reported from a DS patient who had symptoms at birth and died at 3 months of age (3). Our patient had symptoms 13 days after birth. Some typical RMS features of this patient include abnormal glucose homeostasis, hyperinsulinemia, dry skin, thick hair, elevated testosterone and growth retardation. We diagnosed this patient as RMS. With a microdeletion, our patient presents a mild and atypical phenotype. It may be explained by the unclear genotype-phenotype correlation of mutations in \textit{INSR}. Different missense mutations in the same codon relate to different phenotypes (8). One DS patient bearing homozygous deletion of \textit{INSR} and inactivation of IR lived more than two years and died at the age of 3.5 years(9). The coexistence of modifier genes and compensatory pathways may explain the phenotypic variability (10).

The insulin receptor is a tetramer of two \(\alpha\) monomers and two \(\beta\) monomers. It is widely expressed and plays a vital role as a mediator between the extracellular and intracellular insulin signaling pathway. The whole region of the \(\alpha\)-subunit is extracellular. The \(\alpha\)-subunit contains a Leu-rich-compositionally biased region, Cys-rich-compositionally biased region, Fibronectin type-III 1-domain and Insulin in-binding-region. The \(\beta\)-subunit stretches the cell membrane. The extracellular region of the \(\beta\)-subunit contains a Fibronectin type-III 2-domain and a Fibronectin type-III 3-domain. The cytoplasmic region of the \(\beta\)-subunit consists of several functional domains including a juxtamembrane region, a tyrosine kinase domain and the carboxy-terminal-region (5, 11). The \(\alpha\)-subunit is responsible for binding affinity to insulin. The Cys-rich-compositionally biased region is the main binding site of insulin. Fibronectin type-III domains 1 and 2 form the secondary insulin-binding site (5). Deficiency of the juxtamembrane region makes the folding of IR unstable and affects its downstream processing (12). The function of the Fibronectin type-III 3-domain remains unknown. A helical transmembrane region follows the Fibronectin type-III 3-doman. Through binding with insulin, IR motivates phosphorylation of different phosphotyrosine residues in the tyrosine kinase domain (13). Downstream IR substrates bind to phosphotyrosine residues of IR and regulate two main signaling pathways: the PI3K-AKT/PKB pathway and the Ras-MAPK pathway. The PI3K-AKT/PKB pathway is responsible for controlling cell growth and differentiation. The metabolic action of insulin is mainly regulated by the Ras-MAPK pathway (14). 3D structural modeling indicates that with deficiencies in both \(\alpha\)-subunit and \(\beta\)-subunit, IR may be unable to combine with insulin receptor substrates and recruit the downstream signaling molecules (15).

Some unique features of RMS including coarse face, gingival hyperplasia and acanthosis
nigricans do not show in our patient. These three symptoms can be absent in neonates and may develop in adolescence (16). The insulin receptor is expressed in the heart and regulates cardiac cell activity through the phosphatidylinositol 3-kinase (PI3K)-Akt pathway. Insulin receptor deficiency possibly leads to cardiac dysfunction, as observed in patients (10, 17) and proven by animal models (18). There is no clue that indicates any relationship between heart structural malformation and insulin receptors, so we consider that the ventricle septal defect is not a consequence of insulin receptor deficiency. The long-term prognosis of RMS patients is poor (19). Recombinant human insulin-like growth factor 1 and recombinant leptin are recommended for treatment of severe insulin resistance syndrome (19, 20). However, the complications and safety of these drugs remain unknown (21).

In summary, we show an RMS patient carrying one known pathogenic mutation and one novel deletion in \textit{INSR}. Since the presenting clinical features of patients with insulin resistance syndrome can be atypical, when the diagnosis is in doubt genetics testing may help to identify the final diagnosis.

\textbf{Conflict of Interest Statement:} The authors have no conflicts of interest to disclose.

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Figure 1. *INSR* gene compound heterozygous mutation: a known missense mutation and a novel microdeletion. (A). Pedigree of the family. (B). The SNV locates at the tyrosine-protein
kinase catalytic domain and marked by red asterisk. (C). Sanger sequencing shows the mutation is from mother. INSR gene locates at 19p13.3-19p13.2. (D). The deletion fragment is marked within two red lines. This fragment contains Exon 11 and part of Exon 10. (E). Real-time quantitative PCR shows that the deletion is from father.

**Figure 2.** 3D structural modeling of INSR protein. Comparing to the wild type, 3D structural modeling estimates a large portion of deficiency in the monomer form of insulin receptor caused by the deletion. Different domains are marked by different colors. The name of the domain is marked the same color as the domain is.