IDENTIFICATION OF VARIANT OF INSULIN RECEPTOR GENE IN RESISTANT DIABETES

Nguyen Ngoc Khanh✉, Can Thi Bich Ngoc
Vientam National Children's Hospital

Insulin resistance is defined as a reduced biological response of target tissues to normal insulin levels and is a major mechanism leading to type 2 diabetes, particularly in obese individuals. Beyond obesity, other causes include medications (e.g., glucocorticoids, antiretrovirals, oral contraceptives), stress, pregnancy, dyslipidemia, insulin receptor autoantibodies (Type B insulin resistance), and genetic defects. Among genetic causes, mutations in the insulin receptor (INSR) gene can lead to severe insulin resistance known as Type A insulin resistance. This rare inherited disorder belongs to a spectrum of monogenic insulin resistance syndromes, including Donohue and Rabson-Mendenhall syndromes. We report a 12-year-old boy with diabetes due to an INSR mutation. He presented with polyuria, polydipsia, and weight loss. His height was 142cm (0 SD), and BMI decreased from 23.3 to 21.3 kg/m² (> 95th percentile). No acanthosis nigricans was observed. Lab results showed fasting glucose 16.8 mmol/L, HbA1c 7.8%, and HOMA-IR 11.2. Genetic testing revealed a novel heterozygous variant c.4115G>A (p.Arg1372Gln) of INSR gene; the mother carried the same variant, while the father was wild type. The patient responded well to lifestyle modification, insulin, and metformin therapy.

Keywords: Diabetes in children, insulin resistance, *INSR*.

I. INTRODUCTION

Insulin resistance is defined as our body's decreased response to hormone insulin. The clinical manifestation of insulin resistance are characterized by two main groups: those caused by decreased insulin activity and those caused by excessive insulin production. Impaired glucose tolerance, diabetes, growth retardation, and lipodystrophy are the consequences of impaired insulin action on target tissues. Polycystic ovary syndrome and acanthosis nigricans result from an overactive effect of insulin on the ovaries and melanocytes.[1] There are many causes and conditions associated with insulin resistance, among which mutations in the insulin receptor-encoding INSR gene are a group of rare inherited diseases.[2] Depending on the type of mutation, mutation site and inheritance pattern, the disease varies from severe to mild insulin resistance.[3] Donohue syndrome (Leprechaunism syndrome) is the most severe insulin resistance, type A insulin resistance syndrome has milder clinical signs, and Rabson-Mendenhall syndrome presents with moderate symptoms. Donohue syndrome (DS) and Rabson-Mendenhall (RMS) are very rare recessive inherited syndromes. Type A insulin resistance is more common, usually heterozygote and inherited by dominant mutation. The disease generally onsets during puberty time with clear symptoms of insulin resistance. The disease is more common in women than men because hormone problems are more easily detected in women (especially hyperandrogenism). The definitive diagnosis

Corresponding author: Nguyen Ngoc Khanh
Vietnam National Children's Hospital
Email: khanhnn@nch.gov.vn
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is based on the *INSR* gene sequencing to identify the mutation. The *INSR* gene is located on chromosome 19 containing 22 exons and 21 introns. Exons 1 to exon 11 encode for the alpha subunit, and exon 12 to exon 22 encode the proteins making up the beta subunit. After translation, the insulin receptor undergoes glycosylation, folding and dimerization to yield a complete insulin receptor with 2 alpha and 2 beta subunits.[4] To date, there are few reports showing a correlation between the phenotype and genotype of *INSR* mutations and insulin resistance. However, case reports suggested that homozygous or compound heterozygous for mutations on the alpha subunit often cause severe insulin resistance syndromes (DS and RMS), meanwhile mutations found in the beta subunit typically present mild insulin resistance – type A insulin resistance.[5]

Insulin resistance critically requires early diagnosis and treatment. However, the diagnosis remains big challenge to physician. The incidence of insulin resistance in reports is approximately 1/100,000, much lower than the predicted incidence for the disease (1/1,000). In Vietnam, the newly diagnosed cases of type A insulin resistance in children are limited, due to the clinical features are atypical that can be mistaken with other diabetic types, and difficulties in identifying genetic abnormalities related to diabetes. In this study, we report a case of an adolescent with clinical and laboratory manifestations of resistant insulin, genetic test showed mutation in *INSR* gene at the c.4115G>A site (p. Arg1372Gln) of exon 22 coding for the terminal of beta subunit (not previously reported in literature). We analyze the clinical and laboratory characteristics, and the relationship between clinical phenotype and the genetic mutation, so that providing diagnostic approach and notices in treatment for diabetes in adolescents and youth.

II. CASE REPORTS

Clinical manifestation

A 12-year-old boy, admitted to hospital with polydipsia, polyuria and loss weight.

The patient is the third child, normal delivery, full term, birth weight 3.1kg, normal postpartum history. Maternal history: 8kg gain during pregnancy and no screening for gestational diabetes. The boy presented jaundice at 3 days old with no cyanosis or hypoglycemia. Family history recorded that the grandmother was diagnosed with diabetes at 45 years old, passed away at 60 years old, was treated with both oral drugs and insulin injections; the father's aunt was diagnosed with diabetes at the age of 38, is still alive and treated with oral drugs and no insulin.

The disease onset was 2 weeks before admission, the patient presented with polydipsia, polyuria (2 - 3 times during night), with weight loss of 4 kg in 2 weeks. His mother observed presence of ants near his urine. He was taken to the province hospital and identified with high blood glucose condition. He was then admitted to the Vietnam Nation Children's Hospital. On examination: The child was alert, globally obese, height 142cm (0 SD – WHO 2004), body weight 43kg, BMI 21.3 kg/m^2 (previous BMI 23.2 kg/m^2), no breathing difficulty, no dehydration, no acanthosis nigricans. Regular heart rate, clear pulse, normal blood pressure. Genitourinary examination showed clear male genitalia, no pubic hair, penis length was 6 cm, both sides testicular volume was 6ml.

Table 1. Laboratory tests

Test	Unit	Value	Normal range
Glucose	mmol/l	16.8	3.3 – 3.5
Insulin	mU/L	15	3 – 25
C-Peptide	ng/ml	0.936	0.81 – 3.85
HOMA-IR		11.2	< 2
HbA1c	%	7.8	4 – 6.2
Cholesterol	mmol/l	4.2	2.88 – 4.23
HDL-C	mmol/l	1.3	0.9 – 1.79
LDL-C	mmol/l	2.7	≤ 3.3
Triglyceride	mmol/l	0.48	0.51 – 2.38
FSH	IU/L	1.9	1 – 18
LH	IU/L	064	2 – 10
Testosterol	nmol/L	2.1	8.7 – 35
PH		7.40	7.35 – 7.45
$HCO3^-$	mmol/L	23	21 – 26
ICA		Negative	Negative
GAD	IU/ml	0,467	< 1
ZnT8	IU/ml	*43,3*	< 15

Genetic results

He underwent whole exome sequencing, which revealed a heterozygous dominant variant c.4115G>A (p.Arg1372Gln) in the INSR gene (Figure 1).

Diagnosis and treatment

He was diagnosed with Insulin resistance – Diabetes and was prescribed Insulin (0.5 IU/kg/day). After getting the genetic results, he was prescribed Metformin 750mg, twice a day, combined with rapid Insulin only when the blood glucose rises. At 4 months follow-up, he had a stable blood glucose test (6 - 9 mmol/l), HbA1c 7.82%. Subsequently he presented with uncontrolled blood glucose and he was treated with metformin combined with daily injected insulin. The outcome has been stable.

III. DISCUSSION

Insulin resistance is due to a critical defection in insulin signaling pathways in target tissues. One of the essential components of the signaling pathway is the insulin receptor. Mutation in the insulin receptor (*INSR*) gene directly impairs the function of insulin signaling. The insulin receptor consists of two alpha and beta subunits that covalently paired and linked by disulfide bridges.[6] The gene encoding the insulin receptor locates on chromosome 19, compose of 170 kb long and includes 22 exons. Mutant INSR gene induced insulin resistance syndrome was first reported in 1988; Yoshimasa et al described a patient carrying a

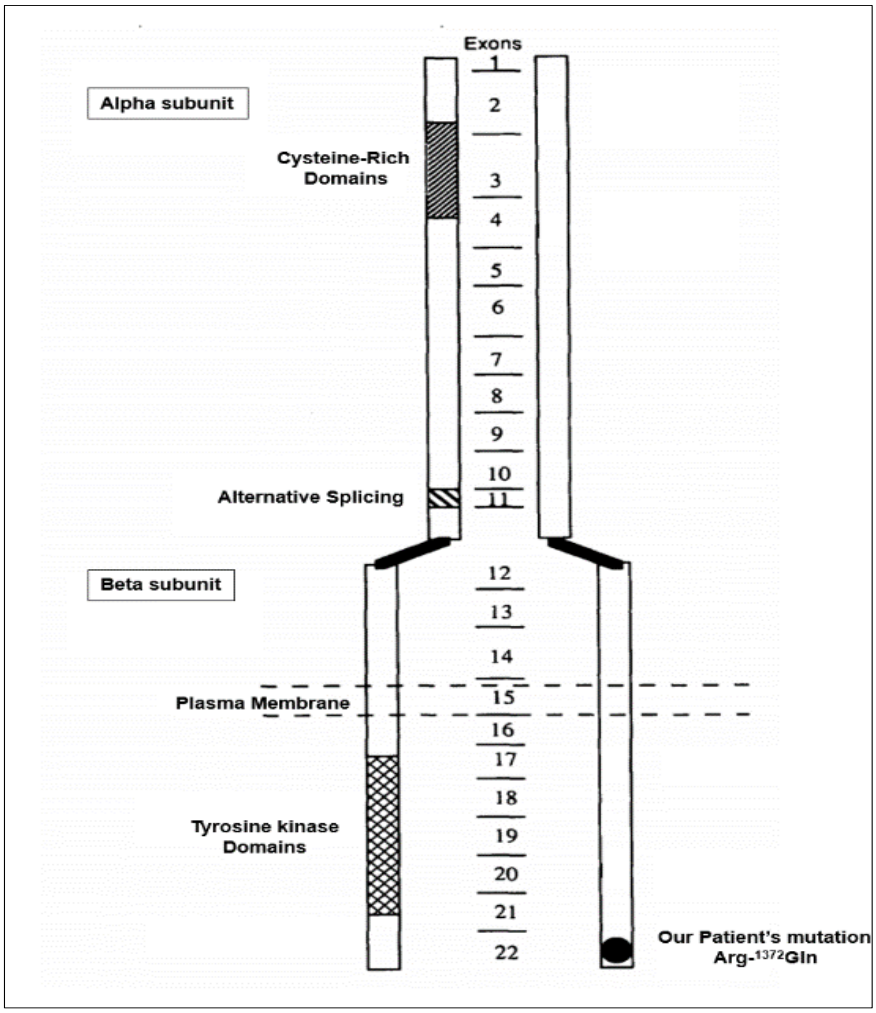

Figure 1. Insulin receptor and the variant of *INSR* gene of the patient

homozygous mutation of the insulin receptor who was diagnosed with Rabson-Mendenhall syndrome.[7] Until now, there have been more than 150 mutations in *INSR* gene identified that causes insulin resistance syndrome. Mutations include missense, nonsense, deletion, addition, and frameshift mutations. The consequences of *INSR* gene mutations on insulin receptor function are classified into 5 groups: (1) Decreased insulin receptor biosynthesis, (2) Abnormal transport of insulin receptors to the cell surface, (3) Decreased insulin binding, (4) Inhibited tyrosine kinase activity, and (5) Enhanced insulin receptor degradation.[8] Type A insulin resistance syndrome is a clinical syndrome commonly caused by *INSR* mutation. The onset disease usually occurs around prepubertal period with symptoms of impaired glucose tolerance, acanthosis nigricans, especially polycystic ovarian syndrome in girls.

In our study, the patient presented with weight loss, polydipsia, and polyuria. These are common manifestation observed in diabetic patients. The onset time was around adolescence and the child began to have signs of puberty. Puberty is a period experiencing

a great change in sex hormones and growth factors that feasible decreases insulin sensitivity.[9] Therefore, the onset of diabetes often occurs during this period. In insulin resistance, the diagnosis time mainly relies on how severe the mutation site has impact on insulin receptor function.[10] Type A insulin resistance patients are characterized by mild insulin resistance syndrome and often have an onset during puberty. Clinical examination revealed that our patient was overweight and did not present with acanthosis nigricans. It is well known that, acanthosis nigricans is one of the typical indicators to suspect of insulin resistance syndrome, which results from the proliferation of keratinocytes and subcutaneous fibroblasts upon the over-stimulating effect of insulin on the IGF-1 receptor. In addition, high insulin levels in the blood stimulate androgen synthesis, which subsequently leads to clinical manifestations such as hirsutism, acne, polycystic ovary syndrome, especially in females. In our case, before coming up with a diagnosis of insulin resistance, we needed to rule out type 1 diabetes due to acute onset in adolescence. We tested insulin level, C-peptide level and autoantibodies associated with type 1 diabetes. The test showed that blood insulin and C-peptide levels were within normal range. Thus, the hyperglycemia at the time of diagnosis was not definitely caused by endogenous insulin deficiency. We then thought of a patient with impaired glucose tolerance due to insulin resistance. This can be partially proved via the insulin resistance index HOMA-IR>10 and HbA1C 7.8%. However, we suspect that the resistance condition is not complete. Our interpretation is that insulin is an important growth factor involved in the process of proliferation, development and metabolism in the body, so that the consequences of complete insulin resistance should include fetal growth retardation, facial abnormalities, and decreased subcutaneous fat.[11] Our patient did not exhibit these symptoms suggesting that his insulin resistance may be partial.

There was another challenge for us during the determination of which type of diabetes in this patient. Immuno-test showed positive result for ZnT8 - an auto-antibodies against zinc transporters on the membrane of insulin granules in the cytoplasm. In type 1 diabetes, anti-ZnT8 autoantibody has a significant diagnostic value. The bioinformatics screening method for genes related to type 1 diabetes showed that anti-ZnT8 antibody is the fourth most important marker contributing to the diagnosis of type 1 diabetes along with GADA, IA2A, and IAA. Among type 1 diabetic patients who were negative for GADA, IA2A, and IAA autoantibodies, 26% of patients were positive for autoantibodies against ZnT8. In other populations, 2% of normal subjects and 3% of patients with type 2 diabetes were positive for ZnT8 antibodies.[12] Thus, anti-ZnT8 autoantibody is an important marker to support the diagnosis of type 1 diabetes. Turning back to our patient, at onset time, the child had elevated blood sugar, but insulin and C-peptide levels were normal, indicating a high probability that the impaired glucose tolerance was not due to insulin deficiency. However, we have not completely ruled out type 1 diabetes because the patient has incomplete insulin resistance, positive anti-ZnT8 autoantibody, onset in the high-risk period. In addition, based on treatment follow-up, the patient had a good response to metformin alone for 4 months, consistent with the diagnosis of insulin resistance. However, in later time, we needed to prescribe combined insulin and metformin to achieve glycemic control goals. Therefore, in order to put a definite diagnosis in this patient, we would continue to

follow up the patient's symptoms and response to treatment.

Based on the initial diagnosis with onset time during puberty, we decided to conduct gene sequence to detect mutation associated MODY. The result showed that the patient carried a mutation in which Guanin was substituted to Adenine at the 4115th nucleic acid position on exon 22 of the *INSR* gene, leads to the replacement of the 1372th amino acid Arginine to Glutamine, and this point mutation belongs to the beta subunit of the insulin receptor. This is a new point mutation in the *INSR* gene that has not been reported before to cause insulin resistance. Exon 22 is the final exon on the INSR gene, encoding 117 amino acids belonging to the carboxyl terminus (-COOH) region of the beta subunit of the insulin receptor. This exon is reported to participate in the formation of the last 3 helices of the tyrosine kinase region, as well as the carboxyl terminus of the beta subunit that catalyzes and binds to nucleotides.[13] This mutation site has been found neither in the mutation database (ClinVar and HGMD) nor in the Asian population mutation database. According to GnomAD all, the mutation rate is estimated to occur at a rate of 0.000229 in the population. Besides, this variant is predicted to be deleterious by In-silico analysis. In the literature, several reports suggest that mutations on exon 22 are associated with insulin resistance syndrome. A review study about identified *INSR* gene mutations reported two missense mutations on exon 22 c.4082A>G (p.Tyr1361Cys) and c.4133G>A (p.Arg1378Gln) caused diabetes and insulin resistance syndrome, respectively. However, there are also other reports suggesting an unclear role for exon 22 in insulin receptor function. An in vitro study made a deletion of the 69 amino acid in the carboxyl terminus of the beta subunit did not alter insulin receptor tyrosine kinase function.[14] In our study, assuming the patient condition is partial insulin resistance, the mutation identified here partially affects the function of the insulin receptor. Analysis of parental genes showed that the mother also carried the mutant gene, with no obvious clinical manifestation. The patient probably inherited the mutant gene from the mother, and this is an autosomal dominant mutation. According to literature, genetic information in type A insulin resistance syndrome can be inherited in a dominant or a recessive pattern; Leprechaunism and Rabson-Mendenhall syndrome mutations are both ecessive.[10] Analysis based on the clinical manifestation of the patient and the mother, we suggested the role of the 1372th amino acid on the beta subunit is not much involved in the signal transduction of the insulin receptor. Besides, the phenotypic variation relies not only on typical genotype, but also on the individual and the environmental factors. Taken together, further research, particularly in vitro study, are needed to draw confirmation about the role of exon 22 as well as the 1372th amino acid on insulin receptor function.

IV. CONCLUSION

We report an adolescent boy presenting insulin resistance caused by a point mutation in *INSR* gene located on exon 22, which has never been reported in the literature. Clinical and laboratory findings clearly implicated a partially insulin resistance syndrome that suggests a role of exon 22 in contributing partially to insulin receptor activity. Therefore, when treating a child with impaired glucose tolerance, clinician should consider the possibility of insulin resistance. Genetic Testing of early onset insulin resistance in young patients should be performed to differentiate insulin resistance in type 2 diabetes.

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