Wolcott-Rallison syndrome due to the same mutation in EIF2AK3 (c.205G>T) in two unrelated families: A case report

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Abstract. Wolcott-Rallison syndrome (WRS) is a rare autosomal recessive disorder characterized by early-onset diabetes mellitus, skeletal dysplasia and growth retardation. Other associated disorders include severe liver and renal dysfunction, and central hypothyroidism. Mutations in the eukaryotic translation initiation factor 2α kinase 3 (EIF2AK3), which is located at chromosome 2p12, are responsible for this disorder. In the present case report, the case of a 3-month old boy diagnosed as neonatal diabetes, who had acute liver failure soon afterwards is detailed. This diagnosis was confirmed through the identification of a novel nonsense mutation in exon 1 of EIF2AK3. The aim of the current case report was to raise awareness for patients with WRS with neonatal diabetes mellitus, particularly those with multiple systemic manifestations.

Introduction

Wolcott-Rallison syndrome (WRS) is a rare autosomal recessive disorder characterized by early-onset or permanent neonatal diabetes mellitus, skeletal dysplasia and growth retardation, and other variable multisystemic clinical manifestations, wherein the majority of patients succumb in infancy or childhood (1-5). Long-term regular insulin therapy has been demonstrated to improve survival rates (6,7). To date, <100 cases of WRS have been reported according to the literature. WRS is mostly reported in individuals from countries where consanguineous families are common (8). It has previously been demonstrated that mutations in the gene encoding eukaryotic translation initiation factor 2α kinase 3 (EIF2AK3) are responsible for WRS (4,5). In the present report, a novel mutation in EIF2AK3 (c.205G>T) was reported in a patient with WRS. Notably, both parents of this patient, who were from unrelated families, were heterozygous carriers for this mutation.

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Case report

The patient was a Chinese boy born at 39 weeks via natural childbirth, with a birth weight of 3.2 kg. His parents were nonconsanguineous. In February 2016, at 3-months of age, the patient was diagnosed with neonatal diabetes mellitus at Wuhan Children's Hospital (Hubei, China). In June 2016, at 7-months of age, the patient was diagnosed with WRS and admitted to the Department of Endocrinology, Zhengzhou Children's Hospital (Zhengzhou, China). The patient presented with vomiting and a high blood glucose level. Experimental treatment with Glibenclamide (1 mg/kg/day; Shanxi YunPeng Pharmaceutical Co., Ltd., Taiyuan, China) was ineffective. The patient was discharged with a regimen of premixed insulin Novolin® 30R (1.0-1.5 U/kg; Novo Nordisk, Bagsværd, Denmark) three times daily.

At 7 months of age, the patient experienced fever of unknown origin, anorexia and vomiting. Anti-infective therapy was ineffective. Physical examinations revealed the following: Height, 65 cm; weight, 7.5 kg. No abnormal physical features, lethargy, or cardiopulmonary abnormalities were observed. The patient's liver was enlarged, extending ~2 cm below the rib cage. Blood glucose was detected using a glucose meter and test strips (Johnson & Johnson, New Brunswick, NJ, USA). The patient's blood glucose levels fluctuated between 2.7-19.0 mmol/l (normal range, 3.9-6.1 mmol/l). Blood C-peptide levels were detected using an electrochemical luminescence method (Roche Diagnostics, Basel, Switzerland). The patient's Blood C-peptide levels were 0.07 ng/ml, which was markedly lower than the normal range (1.1-4.4 ng/ml). Islet cell antibody (ICA) was detected using the indirect immunofluorescent method, while the glutamic acid decarboxylase antibody (GAD) was detected using immunoradiometric methods, according to the manufacturer's protocol (Roche Diagnostics). Both ICA and GAD tests were negative. Renal and thyroid function tests were normal. In addition, chest X-ray, cardiac enzyme levels and electrocardiogram were normal. A liver function test revealed elevated alanine transaminase (ALT) levels of 1,065.9 U/l (normal range, 0-45 U/l) and aspartate transaminase (AST)

levels of 1,887.7 U/l (normal range, 0.35 U/l). An abdominal ultrasound exam revealed an enlarged liver.

The patient received an insulin aspart injection (NovoLog®; Medtronic, Minneapolis, MN, USA) to control blood glucose levels, delivering a large dose (1.0-2.0 units) before meals, with a total of 6 U/day. In addition, the patient also received aspartate ornithine and reduced glutathione injection (150 mg/kg; Qirui Pharmaceutical Co., Ltd., Wuhan, China) to protect liver function, and ceftazidime (300 mg/kg; Hailing Chemical Pharmaceutical Co., Ltd., Hainan, China) twice a day as anti-infection treatment.

Following treatment, the patient's blood glucose fluctuated between 4-9 mmol/l before meals, to 8-13 mmol/l after meals. Although the patient's body temperature and blood glucose levels became steady, the patient's liver function was evaluated, which revealed ALT levels of 6,677.2 U/l and AST levels of 8,976.5 U/l, with systemic edema due to hypoproteinemia. Ultimately, the patient received hemodialysis and was discharged 15 days later. The patient had regular follow-ups for six months, during which liver function was normal.

During hospitalization, blood samples were collected and DNA was extracted from the peripheral leukocytes of the patient and his parents. Written informed consent was obtained from the patient's parents prior to participation in the present case report. The EF2AK3 gene was amplified by polymerase chain reaction, followed by Sanger sequencing performed by Meiji Biomedical Technology Co. Ltd., Shanghai, China. Briefly, blood samples (2 ml) were collected and centrifuged at 13,400 x g for 2 min at room temperature. Subsequently, total DNA was extracted from peripheral leukocytes using the DNA extraction reagent (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocol. To examine the 17 exons of EIF2AK3, DNA was amplified using the TIANamp blood DNA kit (Tiangen Biotech Co., Ltd.) using the specific primer pairs listed in Table I, according to the manufacturer's protocol. The following thermocycling conditions were used for the PCR: Initial denaturation at 94˚C for 5 min; 38 cycles of 94˚C for 30 sec, 62˚C for 30 sec and 72˚C for 45 sec. Sequencing analysis revealed that the patient was homozygous for a novel nonsense mutation, c.205G>T (P.Glu69Ter) in the EIF2AK3 gene (Fig. 1) and both parents were identified as heterozygous carriers for the mutation (Figs. 2 and 3). To the best of our knowledge, this mutation has not been reported previously. Family genealogy of both parents is presented in Fig. 4.

Table I. Primer pairs used for DNA sequencing.

Exon	Primer sequence (5'-3')
1	F: GAGAGGCAGGCGTCAGTG R: CGCGCGTAAACAAGTTGC
2	F: TGAGCATGTGGGATAAGTCC R: TGCCCTAAAGGGACACAAAC
3	F: TCAGGATCAAGACTCCAGCTC R: TGACAACCTCAGGGGAAAAT
4	F: GGAGTTGGTAATCTAACTGATGC R: CCAACAGCAACATTATCTGAA
5	F: GCCCTCTTGTGGCATAAATC R: CTGGGAGAGGAAGAACCGTA
6	F: GCCCTCTTGTGGCATAAATC R: GGCACTCCTGAAGTAGGAAGG
7	F: CCCTCCCTGTTTTTGTTGAA R: GGGCAAAGACAGTCAGGATT
8	F: CTGGGCCATTTGTTTAACTT R: TGAAATTGTCTCCCAAGATG
9	F: TAGTTAAAGACGGGCCTATT R: CAAGAGTAGCTTTGGTGGAG
10	F: AAGACTGGAGGGATAGCAGT R: AGATCTTAGGTCATTTCTTCTTTG
11	F: TGAACTGATTTTCACATTACCAC R: AATTGGCAGCACTTAGAACC
12	F: GCCTTCAGGGTTGTCTTACT R: CATTGTAATCACACAAGCAAA
13 (1)	F: ACAGAGGGTGCAGTTCAGGT R: CACAATGGTTGCCAATATCC
13 (2)	F: AAGGTCAAGGGAGAGAACCT R: ACCCTCTGCTCTCAGATGCTT
14	F: CATGCACACCCACTGTACTT R: CTGGAACACTACTGCCAGTTT
15	F: CTTTGGGATTCAATAATGCT R: CCAATCTGCTGGTATTAAGAA
16	F: TGTGGAATCTGTGGGATGTC R: TGCTAAGGACCGCTTACGTT
17	F: TTTTGCCAGCACTGATTTTA R: TTTCAAGTCTGCAATTTTGG

F, forward; R, reverse.

Discussion

WRS is a rare autosomal recessive disease, which was initially described by Wolcott and Rallison in 1972 (1). It is characterized by early-onset diabetes mellitus, skeletal dysplasia and growth retardation. Other symptoms include severe liver and renal dysfunction, and central hypothyroidism (2-5). The majority of cases have been reported in countries with a high incidence of marriage between first cousins (9). Mutations in EIF2AK3, which is located at chromosome 2p12, are responsible for this disorder (4,5), and encodes for pancreatic endoplasmic reticulum kinase (PERK) (7). PERK is a major endoplasmic reticulum stress transducer in cells. Mutations in EIF2AK3 disrupt the expression and/or function of PERK, which blocks β cell development and impairs gluconeogenesis (6). This gene is highly expressed in the nucleus of pancreatic β cells and bone tissues (10), which comprise the major sites used for diagnosis of this disease. EIF2AK3 is expressed in the liver and kidney at a lower level, which leads to liver and kidney dysfunction, or even liver failure for patients with WRS (10). The prognosis of WRS is poor. Patients typically succumb to hepatic failure and renal failure at an early age (7). However,

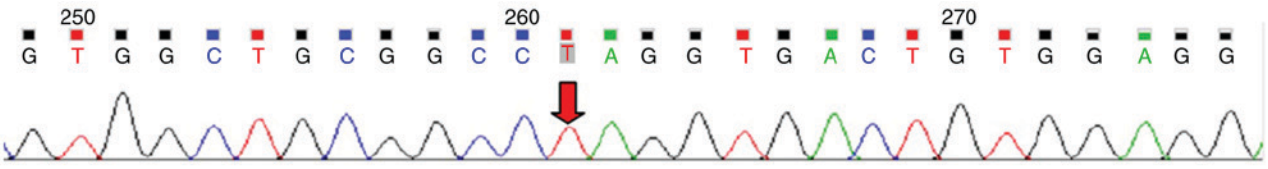

Figure 1. Genetic testing results of the patient.

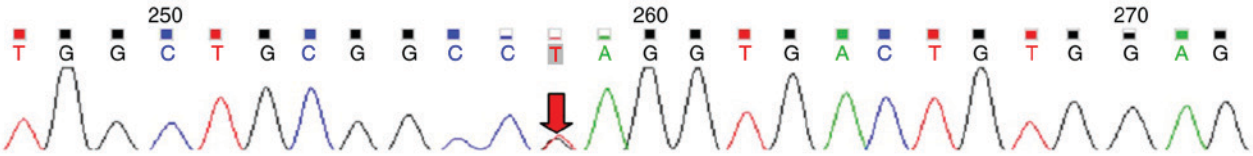

Figure 2. Genetic testing results of the patient's father.

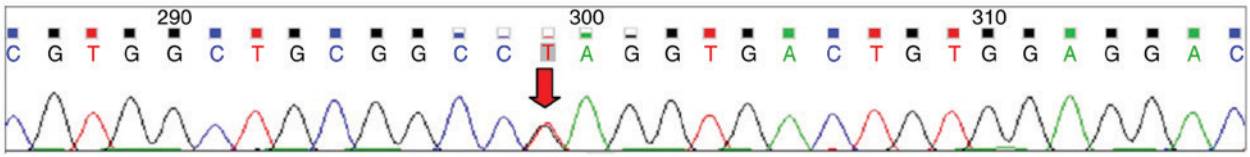

Figure 3. Genetic testing results of the patient's mother.

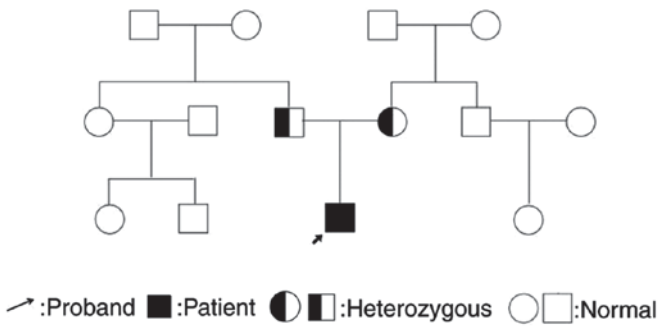

Figure 4. Genealogy of the patient's family.

one patient was previously reported to live until 35 years of age and died without any symptoms (7).

According to previous studies, mutations of EIF2AK3 include nonsense, missense and framework drift mutations, among other types (7,11). In the present case report, the patient was homozygous for the c.205G>T (P.Glu69Ter) mutation of the EIF2AK3 gene which was identified to be a novel nonsense mutation. To the best of our knowledge, this mutation site has not yet been reported in China (6,12). In recent years, other novel mutations in EIF2AK3 have also been reported (13-16). In the present case report, the patient's parents were both heterozygous carriers for the same mutation. Although WRS typically occurs in families with consanguineous marriage (7,10), the present case report details a rare case where the patient's parents are nonconsanguineous, but carried the same mutation site.

The present patient presented permanent neonatal diabetes mellitus and transient liver failure, but no central hypothyroidism or kidney dysfunction, which demonstrates the diversity of clinical manifestations of the disease. It may therefore be hypothesized that this is due to variable expression of the *EIF2AK3* gene, other modified genes, environmental factors and differences in disease management. The patient did not undergo skeletal X-ray, so it could not be determined whether he exhibited epiphyseal dysplasia.

In conclusion, the incidence of WRS is very low. In patients with neonatal diabetes mellitus, particularly those with multiple system manifestations, a diagnosis of WRS should be considered. A detailed history and physical examination may also be beneficial for diagnosis and treatment of WRS. Genetic testing, as the gold standard in diagnosing this disease, may provide guidance for prenatal diagnosis.

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Availability of data and materials

Not applicable.

Authors' contributions

HW contributed to the conception and design. AH and HYW performed the data analyses and prepared the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the patient's parents prior to participation in the present case report.

Patient consent for publication

Written informed consent was obtained from the patient's parents prior to participation in the present case report.

Competing interests

The authors declare that they have no competing interests.

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