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***CDKN1C* gene mutation causing familial Silver–Russell syndrome: A case report and literature review**

Li J *et al.* familial Silver–Russell syndrome

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Abstract

BACKGROUND

Cyclin-dependent kinase inhibitor 1C (*CDKN1C*) is a cell proliferation inhibitor that regulates the cell cycle and cell growth through G1 cell cycle arrest. *CDKN1C* mutations can lead to IMAGE syndrome (*CDKN1C* allele gain-of-function mutations lead to intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita, and genitourinary malformations). We present a Silver-Russell syndrome (SRS) pedigree that was due to a missense mutation affecting the same amino acid position, 279, in the *CDKN1C* gene, resulting in the amino acid substitution p.Arg279His (c.836G>A). The affected family members had an SRS phenotype but did not have limb asymmetry or adrenal insufficiency. The amino acid changes in this specific region were located in a narrow functional region that contained mutations previously associated with IMAGE syndrome. In familial SRS patients, the PCNA region of *CDKN1C* should be analysed. Adrenal insufficiency should be excluded in all patients with functional *CDKN1C* variants.

CASE SUMMARY

We describe the case of an 8-year-old girl who initially presented with short stature. Her height was 91.6 cm, and her weight was 10.2 kg. Physical examination revealed that she had a relatively large head, an inverted triangular face, a protruding forehead, a low ear position, sunken eye sockets, and irregular cracked teeth but no limb asymmetry. Family history: The girl's mother, great-grandmother and grandmother's brother also had a prominent forehead, triangular face, and severely proportional dwarfism but no limb asymmetry or adrenal insufficiency. Exome sequencing of the girl revealed a new heterozygous CDKN1C (NM_000076. 2) c.836G>A mutation, resulting in a variant with a predicted evolutionarily highly conserved arginine substituted by histidine (p.Arg279His). The same causative mutation was found in both the proband's mother, grandmother and grandmother's brother, who had similar phenotypes. Thus far, we found an SRS pedigree, which was due to a missense mutation affecting the same amino acid position, 279, in the CDKN1C gene, resulting in the amino acid substitution p.Arg279His (c.836G>A). Although the SRS-related CDKN1C mutation is in the IMAGE-related mutation hotspot region (the proliferating cell nuclear antigen (PCNA) domain), no adrenal insufficiency was reported in this SRS pedigree. The reason may be that the location of the genomic mutation and the type of missense mutation determine the phenotype.

The proband was treated with recombinant human growth hormone (rhGH). After 1 year of rhGH treatment, the height standard deviation score of the proband increased by 0.93 SDS, and her growth rate was 8.1 cm/year. No adverse reactions, such as abnormal blood glucose, were found.

CONCLUSION

Functional mutations in CDKN1C can lead to familial SRS without limb asymmetry, and some patients may have glucose abnormalities. In familial SRS patients, the PCNA region of CDKN1C should be analysed. Adrenal insufficiency should be excluded in all patients with functional CDKN1C variants.

Key Words: *CDKN1C*; Gene; Silver-Russell syndrome; Mutation

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Core Tip: This is the fourth reported case of familial Silver-Russell syndrome (SRS) caused by a new missense mutation in the PCNA-binding domain of *CDKN1C*. The SRS pedigree, which was due to missense mutation affecting the amino acid position, 279, of the PCNA-binding domain of the *CDKN1C* gene, resulting in the amino acid substitution p.Arg279His (c.836G>A). Five affected family members also showed SRS phenotypes (small for gestational age, proportionately severe short stature, certain facial features (protruding forehead, triangular face, micrognathia), but without limb asymmetry or adrenal insufficiency. Initial efficacy and safety of growth hormone was observed in the proband treated with growth hormone.

INTRODUCTION

Cyclin-dependent kinase inhibitor 1C (*CDKN1C*), also known as p57/Kip2 (OMIM 600856), is active only when inherited maternally. The paternal copy is imprinted on the short arm of chromosome 11 (11p15.4) and is dose-sensitive. By binding to the cyclin/cyclin-dependent kinase complex, the *CDKN1C* protein prevents DNA replication and cell entry into S phase, arrests the cell cycle in G1 phase, and inhibits cell proliferation [1].

Up to 10–15% of cases of Beckwith-Wiedemann Syndrome (BWS) are familial, and most cases are a result of *CDKN1C* loss-of-function mutations [2]. The clinical features of BWS include macrosomia, hyperinsulinemia, and adrenal tumours. In contrast, gain-of-function variants of *CDKN1C* have been shown to cause conditions of growth restriction, including IMGe syndrome (*CDKN1C* allele gain-of-function mutations lead to intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital malformations) [3] and familial Silver-Russell syndrome (SRS) [4].

IMAGe syndrome is characterized by foetal/intrauterine growth restriction, adrenal dysplasia, metaphyseal dysplasia, genital abnormalities, and other characteristics, such as hypercalciuria and hearing loss [5]. Pathogenic single-nucleotide variations in a specific region of the PCNA-binding domain of CDKN1C have been found in children with IMAGe syndrome.

In 2014, Brioude *et al* [4] identified single-nucleotide variants in the PCNA-binding domain (p.Arg279Leu) of CDKN1C in patients with familial SRS for the first time. This type of SRS has a variety of clinical features, including foetal and postpartum growth restriction, particular facial features (triangular face, protruding forehead) and relative macrocephaly but no adrenal insufficiency or limb asymmetry. Two other familial SRSs resulting from mutations in this region have since been reported (p.Arg279Leu, p.Arg279ser) [6, 7]. Inoue *et al* [8] recently examined the genes of 92 aetiology-unknown SRS patients and reported sporadic SRS cases caused by a new CDKN1C mutation, p.Arg316Gln. These cases met the four criteria of the Netchine–Harbison clinical scoring system, but there was no limb asymmetry and no adrenal insufficiency or metaphyseal dysplasia.

Here, we describe in detail a case of familial SRS caused by a new missense mutation in CDKN1C. This mutant gene resulted in an amino acid substitution (p.Arg279His) that was different from previous SRS mutations.

CASE PRESENTATION

Chief complaints

An 8-year-old girl complained of short stature for 8 years.

History of present illness

She was found to be severely short after birth, There was no vomiting, feeding difficulties, dizziness, headache, polydipsia and polyuria. the growth rate was less than 5cm per year.

History of past illness

There was no history of chronic disease.

Personal and family history

The proband, born at 36⁺⁴ weeks' gestational age, was delivered by caesarean section due to foetal hypoxia. Her birth weight was 1.44 kg, her body length was 39 cm (-6.22 SDS), her head circumference was 31 cm, her sitting height was 26 cm, and her head was relatively large at birth. The anterior fontanelle was large (5×5 cm), and the anterior fontanelle was closed at 4 years of age. The proband could crawl at 10 mo, stand alone at 14 mo, walk at 24 mo, and consciously call her mum and dad at 15 mo.

The mother of the proband was 33 years old (IV-2), with an unknown birth history, height 125 cm, weight before pregnancy 18 kg, BMI 11.5 kg/m², head circumference 50 cm, sitting height 69.9 cm, and sitting height/height 0.56. Gestational diabetes was discovered during pregnancy, and she was later diagnosed with diabetes. The proband's grandmother's brother was 58 years old (III-1), with a height of 137 cm, a body weight of 28 kg, a BMI of 14.9 kg/m², a head circumference of 52 cm, a sitting height of 76 cm, and a sitting height/height of 0.56. He was diagnosed with diabetes at the age of 45. The great-grandmother of the proband was 93 years old (I-1), with a height of 134 cm, body weight of 34 kg, BMI of 18.9 kg/m², head circumference of 52 cm, sitting height of 73.7 cm, sitting height/height of 0.55, and no diabetes. All of them had an unknown birth history; however, they all mentioned being very thin and small at birth, and all three of them had a prominent forehead, triangular face, and severely proportional dwarfism but no limb asymmetry or adrenal insufficiency (Figure 1).

The proband's late grandmother was 120 cm tall, and her appearance was similar to that of the proband. She passed away 10 years before due to an accident. She had no genetic testing, but we inferred that she had the same pathogenic mutation based on the genetic pedigree (Figure 2).

Physical examination

The girl had a relatively large head, an inverted triangular face, a protruding forehead, a low ear position, sunken eye sockets, and irregular cracked teeth but without limb asymmetry. She was 91.6 cm tall and weighed 10.2 kg, her head circumference was 48 cm, her sitting height was 54 cm, her sitting height/height was 0.58, and her body mass index (BMI) was 12.1 kg/m². Her motor and language development was normal during treatment. She had no catch-up growth after birth.

Laboratory examinations

Serum insulin-like growth factor 1 (IGF1) was 244.08 ng/mL, adrenal cortex hormone ACTH was 19.3 pg/mL, cortisol rhythm (8 a.m.) was 6.29 µg/dL, blood glucose was 4.13 mmol/L, the growth hormone provocation test (arginine + levodopa) showed a peak value of growth hormone of 29.9 ng/mL. Exome sequencing revealed a new heterozygous CDKN1C (NM_000076. 2) c.836G>A mutation, resulting in a variant with a predicted evolutionarily highly conserved arginine substituted by histidine (p.Arg279His)(Figure 3) .

Imaging examinations

her bone age was 4.6 years, adrenal thin-slice CT and pituitary MRI was normal.

FINAL DIAGNOSIS

Silver-Russell syndrome

TREATMENT

She was treated with recombinant human growth hormone (rhGH).

OUTCOME AND FOLLOW-UP

After 12 mo of treatment, the patient's height was 99.7 cm (-5.9 SDS), her height standard deviation score increased by 0.93 SDS, and her growth rate was 8.1 cm/year (Figure 4). Blood glucose, insulin, thyroid function and IGF-1 Levels were monitored

every three months during treatment. No adverse reactions, such as abnormal blood glucose, were found.

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DISCUSSION

This is the fourth reported case of familial SRS caused by a missense mutation in the PCNA-binding domain of CDKN1C, which was supported by the onset characteristics and genetic test results of the proband and the pedigree. CDKN1C, CDKN1A, and CDKN1B belong to the Cip/Kip family and are cyclin-dependent kinase (CDK) inhibitors [9]. The CDKN1C protein consists of three functional regions: (i) the N-terminal CDK inhibition domain (CdK); (ii) the proline-alanine repeat (PAPA) domain; and (iii) the C-terminal PCNA-binding domain [1]. The C-terminal PCNA-binding domain binds to PCNA, a cofactor of DNA polymerases that encircles DNA and orchestrates the recruitment of factors to the replication fork^[10].

CDKN1C mutations cause diseases with gain-of-function mutations such as IMAGe syndrome [3, 5] and familial SRS [4, 6, 7]. These mutations are located in a small, conserved region of the gene (PCNA-binding domain containing 10 amino acid residues), and the common clinical manifestations of the two include foetal and postnatal growth restriction and forehead protrusion. However, none of the familial SRS patients who have been reported thus far have had adrenal insufficiency or the limb asymmetry that is common in SRS. The PCNA-binding domain is a linear motif required for PCNA-dependent and cbl4cdt2-mediated ubiquitination [11]. The proteins PCNA and CDKN1A associate closely to ensure the gradual ubiquitination and degradation of CDKN1A. The related motifs in CDKN1C are not perfect, resulting in low-affinity binding to PCNA. Low-affinity binding to PCNA is sufficient for monoubiquitination; however, it is not sufficient to carry out the polyubiquitination process required for protein degradation. CDKN1C monoubiquitination may have functions other than protein degradation [10, 12]. Gain-of-function mutations affecting the 279th amino acid have been reported in both IMAGe syndrome and familial SRS (p.Arg279Pro, p.Arg279Ser, p.Arg279Leu) [4, 6, 7]. The Arg279 residue is highly

conserved. However, in a flow cytometry study, the SRS-specific mutation p.Arg279Leu did not affect the cell cycle [4], while the p.Arg279Pro mutation in IMAGE syndrome promoted cell cycle progression^[4]. This finding was consistent with Hamajima's results^[13]. Further research showed that p.Arg279Leu was associated with increased protein stability. These differences in amino acid changes (arginine to proline vs arginine to leucine) may be associated with a differential loss of binding to PCNA. In a recent study in Japan, the genes of 92 clinically diagnosed SRS patients with unknown aetiology were sequenced again. Sporadic SRS cases caused by the CDKN1C mutation Arg316Gln have been found. The clinical manifestations of the patients were consistent with SRS, but they had no limb asymmetry, adrenal insufficiency, or metaphyseal dysplasia. In vitro studies have shown that amino acid substitution leads to increased protein expression in vitro, and increased CDKN1C protein function leads to related phenotypes^[8]. The SRS pedigree mutation (p.Arg279His) reported in this study has not been functionally verified. However, it can be speculated from the above studies that the p.Arg279His mutation increases CDKN1C protein stability.

In a study of IMAGE syndrome, mutations in the PCNA domain impaired the binding of PCNA and ubiquitin ligase to CDKN1C, thereby impairing PCNA-dependent ubiquitination^[3]. Monoubiquitination may have some functions in regulating protein localization, protein interaction and protein chromosome degradation^[14,15,16]; thus, impaired PCNA-dependent ubiquitination might impair other functions of CDKN1C. Accordingly, it can also be speculated that mutations in the PCNA-binding domain may have different effects on ubiquitination, thereby affecting the regulatory characteristics of the domain.

After 1 year of rhGH treatment, the height standard deviation score of the proband increased by 0.93 SDS, and her growth rate was 8.1 cm/year, which was consistent with the first-year rhGH efficacy (growth velocity=8.8 cm/year) of a proband's mother (CDKN1C c.835C>T, p.Arg279Ser) reported by Binder *et al*^[7] and was also consistent with the 1-year height standard deviation increase (0.75 ± 0.44 SDS) on rhGH treatment

in children younger than gestational age [17]. The growth chart of the index patient is presented in Figure 3.

¹ The proband's grandmother's brother (III.2) and mother (IV.1) both had diabetes, in line with the report of Kerns *et al* [18]. They found a variant in a pedigree with short stature syndrome in Ecuador (CDKN1C c.8433G>T, p.Arg281Leu). The affected family members all had intrauterine growth retardation, short stature, and normal adrenal function. Some patients in this pedigree had limb asymmetry, and eight of the 15 affected family members were diagnosed with diabetes before the age of 40.

CDKN1C plays a certain role in the proliferation of pancreatic β -cells. The loss of CDKN1C function leads to enhanced β -cell proliferation. CDKN1C is highly expressed in pancreatic β cells, but its expression is absent in the pancreatic cell hyperplasia foci of infantile hyperinsulinemia patients with silencing of CDKN1C due to the loss of maternal 11p15 somatic cells [19]. Transplantation of short hairpin RNA-induced CDKN1C-silenced human islet cells into mice leads to the proliferation of transplanted β cells [20]. In addition, BWS patients often have hyperinsulinemia, and approximately 50% of BWS patients have hypoglycaemia at birth [21, 22]. A pathology study on the pancreas of four patients with BWS and hyperinsulinemia showed that the endocrine cells of the entire pancreas proliferated significantly, and the BWS-related CDKN1C loss-of-function mutation may be the main precipitating factor of β -cell proliferation [23]. CDKN1C (c.836G>A, p.Arg279His) is a gain-of-function mutation, which may be because this mutation leads to increased protein stability and produces the opposite phenotype from above: decreased β -cell proliferation leads to decreased insulin secretion and the onset of diabetes.

CDKN1C (c.836G>A, p.Arg279Leu)- and (c.836G>A, p.Arg279Ser)-induced familial SRS members have not had diabetes [4, 6, 7]. In this study, the great-grandmother of the proband (I.1) was 91 years old and did not have diabetes. The blood glucose of the proband was normal, but long-term monitoring is needed. Seven of the people with mutations reported by Kerns *et al* [18] (p.Arg281Ile) were also temporarily free of diabetes. All of these mutations were located in the carboxy-terminal region of the "hot

spot" region of the PCNA-binding domain. Kerns *et al* ^[18] demonstrated that the PCNA binding irregularities of p.Arg281Ile variants did not interfere with the ability of this CDKN1C mutant to associate with other proteins, such as the stress-activated protein kinase p38/SAPK, believed to interact with the N-terminus of CDKN1C.

Missense mutations in the highly conserved PCNA binding domain have been associated with clinical phenotypic heterogeneity (from growth restriction to skeletal abnormalities or no adrenal failure or diabetes in early adulthood)^[9]. Further studies are needed to fully elucidate how CDKN1C variants defective only in PCNA binding regions lead to such a wide range of clinical manifestations.

CONCLUSION

In conclusion, gain-of-function mutations of CDKN1C are a rare cause of familial SRS. Its phenotype is similar to that of SRS, but there is no limb asymmetry, and some cases may be combined with abnormal blood glucose. In familial SRS cases, the PCNA region of CDKN1C should be analysed. Adrenal insufficiency should be excluded in all cases with functional CDKN1C variants.

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