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Case Report

Contiguous gene syndrome involving *DAX1* and *IL1RAPL* resulting in adrenal hypoplasia congenita with mental retardation in male child: a case report

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Abstract: A 17-year-old Han male child in China was reported with adrenal hypoplasia congenita and hypogonadotropic hypogonadism associated with mental retardation (intelligence quotient <55). The male child did not present ant history of severe cerebral injury caused by the adrenal crisis. Moreover, the brain magnetic resonance imaging (MRI) showed a normal blood supply to the brain. Biochemical indexes and genes in the blood of the subject were analyzed. The patient harbored mutations with a complete deletion of *DAX1* and *IL1RAPL* genes on the X chromosome. The results demonstrated that this male child did not suffer from mental retardation caused by cerebral injury, and it also alerts us that intelligence-related gene examination should be performed to investigate the mental retardation in patients with adrenal dysplasia without a history of cerebral injury.

Keywords: Mental retardation, adrenal hypoplasia congenita, IL1RAPL, DAX1, contiguous gene syndrome

Introduction

Adrenal hypoplasia congenita (AHC) refers to a rare genetic disorder that was first reported by Sikl in 1948 [1], which can be caused by deletion or mutation of *DAX1/NR0B1* genes (dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome, gene 1/nuclear receptor subfamily 0, group B, member 1) [2, 3]. The incidence rate is estimated approximately 1/12500 in live-born infants [4]. DAX1 plays a key role in the development of adrenal glands, testis, ovary, pituitary gland, and hypothalamus [5]. Classically, DAX1 mutations have been identified in males with primary adrenal insufficiency and HH (hypogonadotropic hypogonadism). AHC is often manifested as early adrenal insufficiency and pubertal gonadal dysgenesis [6]. The present study reported a 17-year-old Han male child in China with congenital adrenal hypoplasia with an intelligence quotient (IQ) <55. The assessment of relevant genes revealed mutations with homozygous in *NR0B1* and *IL1RAPL1* on the X chromosome of the patient; however, no abnormality was detected on *GK* and *DMD* genes. Moreover, these have not yet been reported worldwide. The phenotype of his father was observed to be normal while his mother presented mental retardation, which may be attributed to the deletion of the genes on the X chromosome. Thus, it could be considered that the mutation in the patient was acquired from the mother. However, the parents of the patient did not undergo gene detection, and hence, our evidence was not adequate. Written informed consent was obtained from the patient for the publication of this case report and any accompany images.

Case report

Subject

A 17-year-old male patient was admitted due to 17 years of mucocutaneous pigmentation, which aggravated and was accompanied by nausea and vomiting for 2 days. The patient was a full-term birth, and suffered from mucocutaneous pigmentation of the whole body at birth, with significant pigmentation on the vulva, lips, and the back of the hand, accompa-

Table 1. Symptoms of the patient, main laboratory and imaging findings

Parameters	Indexes of the patient	Normal value
Symptoms		
Age	17	
Skin, and mucosa (**Figure 2A**, **2B**)	Hyperpigmentation	
Weight (kg)	45	
Height (cm)	160	
Penis size (cm)	3*2	
Testicular volume (bilaterally) (mL)	2.5	
Tanner stage	Tanner I stage	
Plasma/serology examination		
Cortisol (8:00 am) (μg/dL)	0.601	8:00 in the morning: 6.2-19.4
Cortisol (16:00) (μg/dL)	20.34	4:00 in the afternoon: 2.3-11.9
Cortisol (24:00) (μg/dL)	2.03	
ACTH (8:00/16:00/24:00) (pg/mL)	491.32/73.16/1092.67	8:00 in the morning: 6-40 4:00 in the afternoon: 3-30 12:00 at night: 0-20
Aldosterone (supine) (ng/L)	61.96	Supine: 30-160
Renin (supine) (ng/ml/h)	36.33	Supine: 0.15-2.33
Angiotensin 1 (supine) (μg/L)	41.1	Supine: 0.15-2.33
Angiotensin 2 (supine) (μg/L/h)	170.9	Supine: 25-60
Sodium (mmol/L)	118.6	135.0-155.0
Potassium (mmol/L)	4.77	3.5-5.5
Glucose (mmol/L)	4.9	3.9-6.1
DHEA (μg/L)		
Dehydroepiandrosterone sulfate	0.5 μg/dL	18-21 years old: 24-537
FSH (base value/peak value) (mIU/mL)	0.65/1.51	1.27-19.2
LH (base value/peak value) (mIU/mL)	0.56/2.82	1.24-8.62
Testosterone (base value/peak value) (μg/L)	<0.1/1.26	2.7-10.6
Progesterone (ng/mL)	0.08	0.10-0.84
hGH (ng/mL)	0.322	0.003-0.971
Estrogen (pg/mL)	5	20-47
Prolactin (ng/mL)	46.42	2.64-13.1
17-hydroxyprogesterone (nmol/L)	0.17	1.8-10.1
Uroscopy		
Urinary 17-OH (μmol/L)	2.1	8.3-33.2
Urinary 17-KS (μmol/L)	4.3	20.8-76.3
Imaging examination		
Enhanced CT of bilateral adrenal glands (**Figure 2B**)	Unclear display in bilateral adrenal glands	
Plain scan of brain MR, enhanced MR of pituitary	No abnormality	

nied by vomiting and dehydration. The phenotype was diagnosed with Addison's disease by another hospital, and a replacement therapy of hydrocortisone acetate (5 mg q.d.) was administrated. Consequently, the mucocutaneous pigmentation was alleviated, and symptoms of nausea and vomiting were relieved. However, the motor development of the patient lagged behind his peers. For example, he could raise his head at 5 months, sit at 9 months, crawl at 1.5 years, speak a single letter at 3 years of age (continue to be inarticulate until now), and could walk at 5 years old. In the recent 4 years, his penis and testicles were found to be small without secondary sexual characteristics, and his growth was slow at a rate of 2-3 cm/year.

Following, the patient was treated in another hospital. Hydrocortisone acetate was adjusted to be administrated orally: 10 mg in the morning and 5 mg at noon. Subsequently, the dosage of hydrocortisone acetate was readjusted 1 year ago, and 20 mg was administrated orally in the morning, intermittently. Two days ago, the patient presented repetitive coughing and sputum with nausea and vomiting as a result of cold infection and was emergently admitted.

Family history: The parents were non-consanguineous marriage, and the patient was the only progeny. The father was healthy while his mother exhibited mild mental retardation (IQ could not be measured due to unavailability).

Physical examination: The following indexes of the patient were measured: body temperature, 36.3°C; pulse, 90 beats/min; breathing, 20 times/min; blood pressure, 110/78 mmHg; height, 160 cm (-2SD); body weight, 45 kg; upper limb, 76 cm; and lower limb, 88 cm. The patient was tall, thin, with a clear mind, and weak spirit. Significant mucocutaneous pigmentation could be found on the whole body; the joints of fingers and toes, lips, and elbow were the most important. He was devoid of hair-growth around the lips, armpits, and pubes. The thyroid was not big, and the sound of lung breath was coarse without moist rale. The heart rate of the patient was 90 beats/min, and the cardiac rhythm was regular with healthy heart sounds. The thickness of the abdominal fat was 1.0 cm, and the abdomen was soft. However, the liver and spleen were not examined. The result of the examination of the external genitals is shown in **Table 1**.

Laboratory testing: The following indexes were normal, including liver and kidney function, triglycerides, muscular enzymes, and thyroid function. The karyotype was 46, XY.

Materials and methods

Functional test

For the luteinizing hormone-releasing hormone (LHRH) stimulation test, 100 μg Decapeptyl (Ferring, Germany) was injection intramuscularly, and a blood sample was collected to measure the LH and FSH levels after 0, 15, 30, 45, 60, and 90 min, respectively. For the human chorionic gonadotropin (hCG) stimulation test, a single dose of 5000 U hCG (Livzon Pharmaceutical Group Inc., Zhuhai, China) was injected intramuscularly, and a blood sample collected to measure the T level at 0, 1, 2, and 3 days, respectively. Then, the pituitary and testicular functions were evaluated. The base and peak values of LH, FSH, and T are shown in **Table 1**.

Genetic detection

After obtaining the informed consent, 2 mL of peripheral blood was collected from the subject, and genomic DNA was extracted. The following steps were performed sequentially, including the construction of genomic DNA library, the enrichment of the target DNA segments (Agilent, USA), high-throughput sequencing (Illumina, USA), and analysis of the sequencing data using specialized bioinformatics software. Subsequently, the routine analyses were performed, including SNP, InDel, and copy number variation. However, FISH analysis and methylation analysis of DAX1 was not conducted due to its cost-ineffectiveness.

Results

Clinical diagnosis

The patient presented typical symptoms of adrenal insufficiency, which were manifested as HH, low serum cortisol, high ACTH, low serum testosterone, low DHEA (dehydroepiandrosterone), low FSH, low LH, the flat peak of GnRH stimulation test, and hCG stimulation test after entering into adolescence. Thus, he was highly suspected for AHC.

Genetic examination

Subsequent to second-generation sequencing, SNP, InDel, and copy number mutation analysis found deletion mutations in *IL1RAPL1* and *NR0B1* genes on X chromosome, at a molecular weight of 1.52 Mbp (the deletion interval was chrX: 28807411-30327496) (**Figure 1**).

The deletion mutation of *IL1RAPL1* and *NR0B1* genes can adequately explain the clinical symptoms of mental retardation and adrenal insufficiency, which further confirmed that the patient suffered from adrenal hypoplasia congenita with a mental disorder caused by deletion of the *IL1RAPL1* gene.

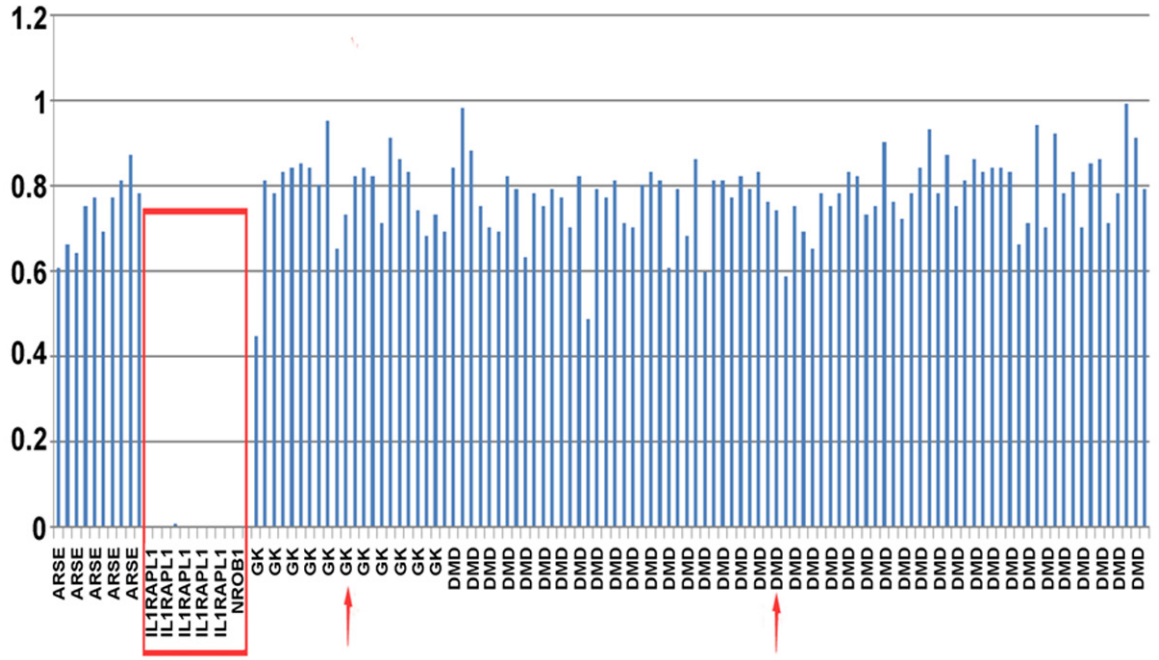

Figure 1. Results of gene-trap and high-throughput sequencing of the patient. Note: The abscissa is the positional information of each locus of the target area while the ordinate is the corresponding coverage of each position. The submitted samples displayed a deletion mutation on the X chromosome, and the interval was chrX: 28807411-30327496. The mainly involved causative genes were *IL1RAPL1* and *NR0B1* (marked by red square). No abnormality was found in *GK* and *DMD* genes (pointed by red arrows). (NR0B1 gene was completely deleted, including two exons and one intron).

Treatment: For the treatment of the decreased adrenal function, the patient was administered a physiological dose of glucocorticoids. After 3 months, the skin color of the patient faded, and cortisol was normal, whereas ACTH was reduced. For the treatment of reduced sexual function, a replacement therapy of testosterone undecanoate was given, which normalized the serum testosterone of the patient.

Discussion

The genetic phenotype of adrenal hypoplasia congenita is associated with X-linked recessive heredity or autosomal recessive heredity [1]. Four clinical forms of AHC have been described: 1) a sporadic form, associated with hypoplasia of the pituitary gland, characterized by small adrenal glands, and a reduction of the fetal zone; 2) a recessive autosomal form with miniature adult adrenal morphology; 3) an X-linked cytomegalic form associated with HH; 4) an X-linked form linked to glycerol kinase deficiency and/or Duchenne muscular dystrophy (DMP) [5, 7]. The gene related to XL-AHC is located in band 1 of zone 2 of the short arm of the X chromosome (Xp21), which is known as dosage-sensitive sex reversal-congenital adrenal hypoplasia gene 1, or *DAX1* (*NROB1*) [8, 9]. *DAX1* gene expresses in the hypothalamus, pituitary, adrenal glands, and gonads, and its mutation can result in the deficiency of gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus and/or LH and FSH secreted by pituitary [5, 10, 11]. DAX1 protein plays a key role in spermatogenesis of Sertoli cells, and the deletion of *DAX1* gene can lead to the occurrence of HHG [12, 13]. A variety of studies have confirmed that pubertal development is delayed in the male children with *DAX1* genetic mutation or deletion, and their secondary sexual characteristics are also impeded. The levels of LH, FSH, and testosterone were low in case of our patient, which also confirmed the above theory. However, the testicular testosterone function was low as assessed by the hCG stimulation test, which was consistent with the study of Tamai and Iyer who found that the rats with *DAX1* gene deletion had detrimental testicular development, secretory dysfunction, and dyszoospermia that resulted in infertility [7, 14, 15].

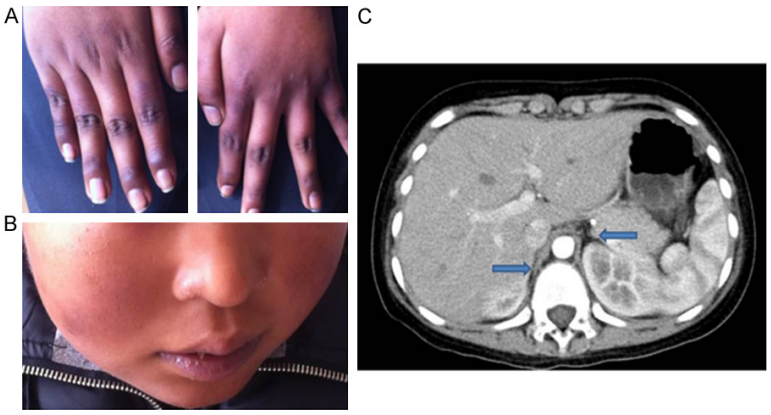

Figure 2. A, B: The patient showed significant mucocutaneous pigmentation. C: Enhanced CT scan of bilateral adrenal glands was unclear.

AHC is difficult to be differentiated from salt-losing adrenal hypoplasia congenita (CAH) in terms of the age of onset, clinical manifestations, and biochemical tests. However, CT examination of the adrenal glands can aid in the differentiation and diagnosis. Most CAH patients have adrenal hyperplasia, but the manifestation of AHC is mal-development of the adrenal cortex. The manifestations of CAH patients are hypersecretion of the sexual hormones, such as increased testosterone, lengthening of the male penis, and masculine women while the AHC patients have normal or reduced manifestations. Sick children surviving to adolescence show varying degrees of gonadal dysgenesis of hypogonadotropic [16]. In addition, ACTH stimulation trial can be helpful in discerning AHC. After ACTH stimulation, no change in the serum cortisol, urinary-17 hydroxy-cortico-steroid, and 17-ketosteroide in AHC patients was seen. However, in CAH patients, urinary-17 hydroxy-cortico-steroid does not respond, or the response is lower than normal, whereas 17-ketosteroide increases. Adrenal CT of the patient showed that the adrenal glands were mal-developed, and testosterone and dehydrogenation of testosterone of its precursors reduced, which could be easily distinguished from CAH.

In summary, laboratory examination of the patient found low plasma cortisol, high ACTH, high renin activity, low sodium, and high potassium, but without the accumulation of 17-hydroxyprogesterone, DHEA, and other steroid precursors. The imaging manifestation did not distinctly reveal the bilateral adrenal glands (**Figure 2**). Combining with the deletion of *DAX1* gene, the diagnosis as AHC was definite. However, considering that except adrenal hypoplasia congenita and HH, the patient had obvious mental disorders, without a history of hypoglycemia caused by adrenal insufficiency and cerebral injury caused by the adrenal crisis. Since *DAX1* gene was located on Xp21, XL-AHC may be considered a symptom of deletion syndrome of the neighboring genes around Xp21. Thus, we obtained information on the homozygous deletion of *IL1RAPL1* and the neighboring genes around *NR0B1* by manual analysis of the CNV. *IL1RAPL1* [17, 18], and X-linked heredity can result in mental retardation and reduce the learning ability of the patient, thereby, illustrating the mental disorders of the patient, consistent with the clinical manifestations. The analysis of the sample discovered that the deletion of *IL1RAPL1* and *NR0B1* genes needed to be identified with the deletion syndrome of neighboring genes around Xp21. Xp21 contiguous gene deletion syndrome is also referred to complex glycerol kinase deficiency (CGKD) [19], which represents the deletion of different sizes of segments of loci containing glycerol kinase in the Xp21 region of the chromosome [20], usually involving the loci of the three disease genes of AHC, GKD, and DMD [21]. However, the diseased child reported in our study presented only the clinical performance of XL-AHC and mental delay, without that of myasthenia gravis and serum triglyceride; muscular enzymes were normal. In addition, his symptoms, signs, and relevant laboratory tests did not show performances of GKD and/or DMP. Hence, it was not considered to be CGKD but continued to be considered as mental retardation caused by adrenal hypoplasia congenita combined with the deletion of *IL1RAPL1* gene.

The manifestation of AHC is primarily the mutation of *NR0B1* gene, and combined deletion of *NR0B1* and *IL1RAPL1* genes is rare. In the current case, AHC was caused by the combined deletion of *NR0B1* and *IL1RAPL1* genes, which was in agreement with the report by Sasaki et

al [22]. However, the patient in their report only had submicroscopic interstitial *DAX1Xp* deletion involving *DAX1* and disrupting *IL1RAPL1*, and the mother heterozygous for the *Xp* deletion was apparently free from mental retardation, under random X-inactivation. In our case, the mother of the patient had mild mental retardation, which was also similar to the study of Muroya, et al [23]. Moreover, we could not perform the analysis of family genes because of the unavailability of the mother of the patient.

These data indicate that the molecular analysis of *DAX1* (*NR0B1*) and *IL1RAPL1* genes is critical for the diagnosis and genetic counseling of children with primary adrenal insufficiency. Especially for the patients with clinical manifestations of AHC combined with delayed mental development, relevant genetic testing is essential.

Disclosure of conflict of interest

None.

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