P3-012

Clinical follow-up of an Adrenal Hypoplasia Congenita patient with a novel mutation in NR0B1

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INTRODUCTION

Gene disturbances of NR0B1 (nuclear receptor subfamily 0, group B, member 1) mediated by the point (missense) mutation, frameshift mutation, nonsense mutation, and/or gene deletions is directly associated with the adrenal hypoplasia congenital (AHC) disease and hypogonadotropic hypogonadism. NROB1 (DAX-1) gene located on the short arm of the chromosome X (Xp21) classified as a dosage-sensitive sex reversal gene associated with the X-linked adrenal hypoplasia. Alike the NROB1 gene, the SF-1 (NR5A1) gene on the X chromosome plays a significant role in the human adrenal, pituitary, and gonadal development. Since the discovery of the NROB1 gene in 1994, numerous mutations had been recorded on this gene. As per the information procured from the Human Gene Mutation Database （HGMD） database, till April 2018, there are 255 mutations (in total) have been identified with the NROB1 gene. NROB1 (DAX-1) gene receptor protein that is derived from the orphan nuclear receptor superfamily is abundantly expressed in the hypothalamus, pituitary, adrenal and gonadal region. NROB1 (DAX-1) gene protein expression markedly regulates the synthesis of steroid hormones and its associated organ development. Concertedly, the NROB1 gene-protein, and the steroidogenic factor-1 (SF-1) protein mediate the differentiation and development of the adrenal cortex and reproductive axis.

CASE PRESENTATION

A young male boy aged about 5 yrs and 9 months old was admitted in the Department of Endocrinology and Metabolism, Zhengzhou Children's Hospital in the year 2007 with these noticeable clinical symptoms: an emerging skin hyperpigmentation; penis enlargement; enhanced growth development for 2 years; fatigue; drowsiness; appearance of pubic hair for 2 months. Our investigation confirmed that the patient hasn't had any signs of such clinical symptoms during his early childhood. Also, the patient didn't show any signs of other adverse clinical symptoms such as seizure, fever, vomiting, coughing, hypoglycemia, and/or abnormal salt craving during his childhood days. The patient's parents were diagnosed to be healthy with a normal growth development with the mean height measured about 176 cm of the biological father and about 162 cm of the biological mother with no prior-history of genetic abnormalities or hypoglycemia. The patient's younger female sibling also examined to be healthy with the normal growth stature.

During the time of admission, the patient was measured with the height about 126.2 cm and weighed about 22kg with the systolic/diastolic blood pressure measurements 90/60 mmHg. The patient had a strong physique with the well-proportioned body structure in concorded to the moderate nutrient intake. Despite these improved clinical or phenotypic characteristics, the patient showed adverse skin symptoms that comprised of fragile skin elasticity, and a disoriented skin pigmentation markedly noticed at the ear edges, lips, arms, nipples, penis and scrotum. Additionally, a darkened skin pigmentation was spotted on the patient's lower limbs. Without showing any other unusual phenotypic or clinical symptoms like a beard growth, thyroid enlargement, or larynx development, the patient was diagnosed with the bilateral breast development (at the B1 stage) with the noticeable pigmentation at areola. The cardiopulmonary abdominal examination showed normal in the patient. Moreover, the patient showed a remarkable enlargement in the penis growth (6.5cm×1.8cm), and had a sparse pubic hair growth with the soft, bilateral testis measured about 3 ml in volume. Perianal pigmentation was also detected during the clinical examination.

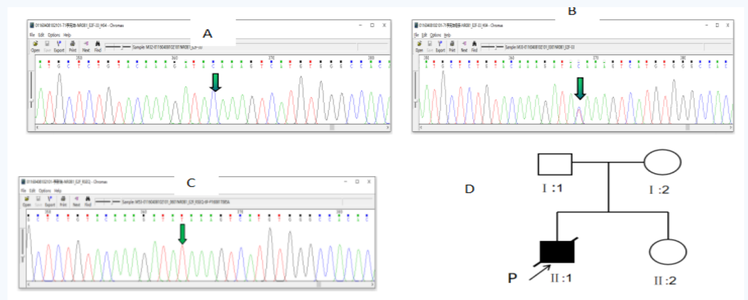
Laboratory and Imaging Evaluations

Lab study		Results	Reference range
Blood gas	PH	7.282,	7.35-7.45
	BE	-8mmol/L	-3 - +3
Sodium		125 mmol/L	135-155mmol/L
Potassium		5.15 mmol/L	3.5-5.0mmol/L
ACTH		192pg/mL	24-80 pg /mL
Cortisol		25 ng/mL	40-82ng/mL
Testosterone		0.43 ng/ml)	<0.025ng/ml
17-hydroxyprogesterone (17-OHP)		0.69 pg/ml	0.07-1.53pg/ml
Luteinizing hormone (LH)		0.43mIu/L	
follicle-stimulating hormone (FSH)		0.9 mIu/L	
GnRH stimulation test 60min	LH	38.72mIu/L	
	FSH	55.41mIu/L	
estradiol (E2)		37.83 pg/ml	0-5ng/ml
24 h urinary free cortisol		3.55 ug/24h	30–350 ug/24h
bone age(BA)		9years old	

gene analysis

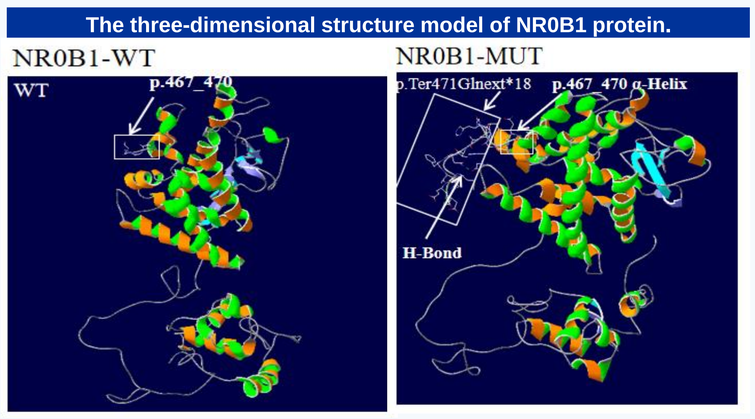
According to the Next-generation gene sequencing,the patient had a NR0B1 gene exon 2 c.1411T>C (p.X471Q) hemizygous mutation, which is a non-stop mutation and has not been reported previously. It has been reported in the HGMD database that a c.1411T>G (p.X471E) hemizygous mutation at the same amino acid position is pathogenic,and according to ACMG the mutation is likely pathogenic, so the mutation we identified is likely to be pathogenic too. As expected, the mother of the patient was found to carry the NR0B1 gene c.1411T>C heterozygous mutation. The father and younger sister of the patient had no related mutations. These findings agree with the X-linked recessive inheritance of the disease

The part picture of NROB1 gene about the patient and his mother/sister



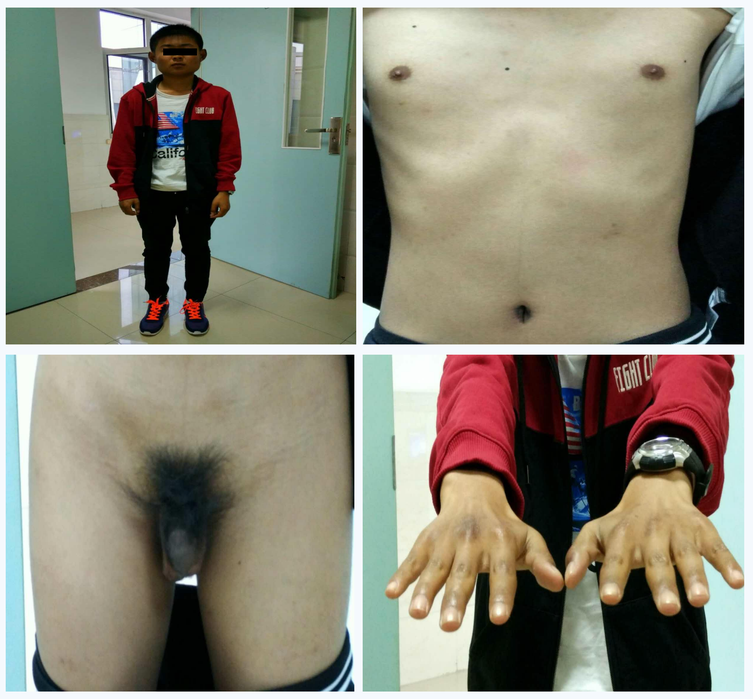
A:The C.1411T > C was detected in the child
B:The C.1411T > C heterozygous mutation was detected in his mother
C:No C.1411T > C heterozygous mutations was detected in his sister
D: Pedigree of the family

The three-dimensional structure model of NR0B1 protein.



Adding 17 amino acids at the end of the protein may affect the conformation of the protein by forming hydrogen bonds and changing the secondary structure of amino acids.

The pictures of the boy



From the pictures,we can see the skin pigmentation markedly noticed at the ear edges, lips, arms, nipples, penis and scrotum.Also we can see the Tanner stage.

follow up

The follow up of the patient with AHC to DAX1 mutations and precocious puberty in this paper.

	Age（y）					
	5.9	11	11.8	12.3	13.1	15.1
Clinical findings						
Height（cm）	126	151	153	154	155	156
Weight（kg）	22	38	39	39	39	40
Hyperpigment	++++	++	++	++++	+++	++++
Pubic hear(Stage)	PH2	PH4	PH4	PH5	PH5	PH5
Penis（cm）	6.5	7.5	7.5	8	8.5	8.5
Testis（ml）	3	3	3	3	3	3
BA（y）	9	14	14	15	16	18
Serum concentrations						
ACTH	192	48	110	162	>2000	786
COR	25	98	56	45	61	48
17-OHP	0.69	0.24	0.46	0.49	1.61	2.16
DHEAS	/	/	21.5	/	3.14	15.5
LH	0.43	3.43	1.78	2.35	3.63	1.93
FSH	0.9	21.8	19.5	22.3	18.4	21.8
T	0.43	2.35	1.93	2.24	3.87	5.65
E2	37.8	21.1	18.2	25.6	41.2	29.6
Medication dosages						
HC（mg/d）	20	20	30	30	/	10
FC（mg/d）	0.05	/	/	/	0.1	0.1
DEX(mg/d）	/	/	/	/	0.375	0.5

DISCUSSION

We report the unique clinical presentation of a boy with AHC who has a point mutation of the DAX-1 gene. Based on the child's clinical manifestation, especially precocious puberty, primary adrenal insufficiency during the early stages, the child was misdiagnosed with adrenal gland syndrome, of which the most common disease is congenital adrenal hyperplasia caused by the loss or decrease of certain enzyme activity that is essential for the synthesis of adrenocortical hormone. The low blood pressure and low blood sodium in the initial diagnosis are unlikely caused by 11-β hydroxylase deficiency and 17-a hydroxylase deficiency. Therefore, it is possibly caused by 21-hydroxylase deficiency, although the adrenal gland-derived sex hormones, including 17-OHP and DHEAS remained at a lower level.

Meanwhile, it was found during follow-up exams that LH and FSH in patient increased rapidly after HC treatment, as well as in response to gonadotropin stimulation challenge, suggesting that the hypothalamic-pituitary-gonadal axis is highly responsiveintact. However, the size of testicles was not increased.

Considering that the level of AMH and inhibin B levels were lower than normal, these results indicated that testicular sertoli cells had functional defects. Then the next generation sequencing performed on the isolated peripheral blood-DNA samples of the patient has confirmed the novel missense mutation with the heterozygous c.1411T>C (p.X471Q) occurred at the NR0B1 gene exon 2.This novel NR0B1 gene mutation was also confirmed by the Sangers DNA sequencing.This site mutation has not been reported previously in the International Human Gene Database and literatures. This new nonstop mutation, which causes the stop codon to be mutated to glutamine, leads to the encoded DAX1 protein to be elongated and potentially nonfunctional. This mutation occurs in the ligand binding domain of the protein and might have a great impact on protein functions. It has been reported previously that a same site mutation to glutamate is pathogenic.Furthermore, a follow-up of the patient's clinical manifestations and laboratory examinations have confirmed the incidence of AHC,a X-link recessive genetic disorder in the patient.

Studies have shown that NR0B1 gene encodes a protein of 470 amino acid residues. Mutations in the NR0B1 gene can lead to developmental disorders of the adrenal cortex, decreased synthesis of steroids, defects in hypothalamic gonadotropin-releasing hormone cells and pituitary cells that produce LH and FSH, and testicular dysplasia. Moreover, different mutation sites can lead to different clinical phenotypes, and even the same site mutation can lead to different clinical manifestations. There are inconsistencies between genotypes and clinical phenotypes and The disease can occur in infancy, childhood or even adulthood; the clinical symptoms can be either serious salt-losing or a simple low gonadotropin-induced sexual dysplasia. The disease can be clinically misdiagnosed as primary aldosterone deficiency, Addison's disease or even 21-OHD etc. Studies have shown that a certain genotype is not necessarily associated with certain phenotypes and the exact pathogenesis remains unclear. Some researchers proposed that the impact of environmental factors on the function of mutated DAX1 protein might be the cause of multiple clinical phenotypes.

Recently, many reports have shown that children with AHC have precocious puberty[17-20].The clinical manifestations of CPP are mostly evident within 1 year of age. After appropriate hydrocortisone and FC replacement therapy, most of the PP symptoms are relieved. In a few cases, the PP symptoms were mitigitated by cyproterone or leuprolide treatment and HH appeared during puberty. Two cases with PPP were treated with high-dose hydrocortisone and the symptoms were mitigitated after 4 doses. In our case, high-doses of hydrocortisone (20 mg/m^2/d), in combination with dexamethasone after bone closure, were used; however, the symptoms of precocious puberty progressed rapidly in an uncontrollable manner.

The underlying causes of precocious puberty remain unknown. Possible mechanisms include: (1) NR0B1 has been shown to regulate the development of primitive stem cells by inhibiting cell differentiation. Defects in NR0B1 gene can lead to the early differentiation of stem cells into immature sterol cells, which leads to the early excessive secretion of hormones, such as ACTH, LH\FSH etc, followed by cell failure. The cells expressing mutant NR0B1 exhibit an age-dependent decline in functions, which can explain early precocious puberty followed by HH. (2) NR0B1 inhibits the reproductive axis before puberty, and its loss-of-function leads to disinhibition which may be related to CPP . But this does not explain the spontaneous remission of precocious puberty. (3) There are a large number of chromophobe cells in the middle section of pituitary that can differentiate into cells such as ACTH, LH, FSH-releasing cells. It remains unknown if NR0B1 has any regulatory effect on the differentiation of these chromophobe cells. (4) it is known that melanocortin-stimulating hormone (MSH) receptor exist on testicular Leydig cells. ACTH can bind to MSH receptors and activate Leydig cell to secrete a large amount of testosterone, leading to ACTH-dependent peripheral precocious puberty. Hydrocortisone treatment can inhibit ACTH secretion and thus reduce testosterone secretion.

The clinical manifestations of the boy patient in this case may be caused by the factors mentioned above. The decreased levels of LH and FSH may be related to the long-term increased plasma level of ACTH. Meanwhile, the bone age has reached 11 years old. After treatment, secondary central precocious puberty was observed. Because of the absence of timely follow-up and treatment with gonadotropin-releasing hormone analogues, precocious puberty was not mitigated for a long period of time. Except for testicular dysplasia, the hypothalamic and pituitary functions of this child patient were not affected. Further follow-up and examinations are needed to assess if the LH- and FSH-secreting cells will gradually deplete with age. Adolescent induction therapy has been applied to AHC patients, however, no improvement in spermatogenesis was observed. This suggests that NR0B1 gene mutation leads to impaired reproductive axis with HH and primary spermatogenic disorders. The main culprit for the impaired testicular development might be that the damages of sertoli cells and the seminiferous tube are so severe that the testicular interstitial cells can no longer support spermatogenic process.

The appropriate treatment and close monitoring of AHC are critical for the clinical outcome. Many studies showed that, with early diagnosis and treatment, AHC children can grow to normal height. Because of the lack of regular clinic follow-up and medication, the patient in the current case had only grown to a height (156cm) that is far below his genetic target height (175.5cm). The child has developed self-inferiority that directly affects his quality of life.

CONCLUSIONS

We report a point mutation of NR0B1(c.1411T>C（p.X471Q)) that add 17 amino acids at the end of the protein which may affect the conformation of the protein by forming hydrogen bonds and changing the secondary structure of amino acids. The mutation of NROB1 gene can lead to a wide range of clinical phenotypes. Besides the classic type of primary adrenal insufficiency, low gonadotropin-related sexual dysplasia, and impaired spermatogenesis etc., the clinical phenotypes can also be precocious puberty, especially in infants and young children. The difference between NR0B1-related AHC and 21-OHD should be noted and early treatment and regular follow-up are essential for clinical outcome.