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A Novel NR0B1 Gene Mutation Causes Different Phenotypes in Two Male Patients with Congenital Adrenal Hypoplasia*

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Summary: X-linked congenital adrenal hypoplasia is characterised by the acute onset of primary adrenal insufficiency in infancy or early childhood and hypogonadotropic hypogonadism (HH) at puberty, arising from mutations of the nuclear receptor subfamily 0 group B member 1 (NR0B1) gene. This study investigated an extended family with two affected males (patient A: 23 years and patient B: 2 months old) and three carrier females. Sequencing analysis of the NR0B1 gene coding region from the family revealed a novel hemizygous deletion [c.604delT; p.(C202Afs*62)] in the two male patients. Furthermore, the patients' respective mothers and their common grandmother had this heterozygous mutation, but it was not present in the Human Gene Mutation Database. The two male patients showed inconsistent clinical features at onset, particularly in early childhood; however, it is possible that the younger patient will eventually show a delay of puberty, feminisation, and nonspermatogenesis in adulthood, similar to that in the older patient. Identification of a novel NR0B1 mutation in this family is important for the diagnosis and genetic counselling of children with primary adrenal insufficiency and HH, and will be helpful for predicting long-term clinical symptoms.
Key words: nuclear receptor subfamily 0 group B member 1 gene; hypogonadotropic hypogonadism; X-linked adrenal hypoplasia congenita

X-linked adrenal hypoplasia congenita (AHC) is a rare disorder that is characterised by primary adrenocortical failure, due to lack of the permanent adult cortical zone of the adrenal glands[1]. Patient symptoms often present as adrenal insufficiency occurring in early infancy or childhood including salt loss, vomiting, a prolonged period of postnatal jaundice, skin hyperpigmentation, hyponatraemia, hyperkalaemia, low cortisol levels, low aldosterone levels, and high levels of adrenocorticotropic hormone (ACTH). In addition to adrenal insufficiency, hypogonadotropic hypogonadism (HH) is a frequent feature of X-linked AHC that is usually recognised during adolescence by the absence or interruption of normal pubertal development[2].

AHC is caused by deletions or point mutations in the nuclear receptor subfamily 0 group B member 1 (NR0B1) gene in the dosage-sensitive sex reversal, adrenal hypoplasia critical region on chromosome X (DAX1). NR0B1, located on the short arm of the X chromosome (Xp21.3-p21.2), encodes an orphan nuclear hormone receptor that functions as a transcriptional regulator of other genes[2]. The NR0B1 gene is crucial for proper development and function of the adrenal glands, gonads, hypothalamus, and pituitary gland, and disruption of the gene can result in various clinical phenotypes. Nearly 200 mutations in the NR0B1 gene have been identified to date, and the relationship between mutations of the NR0B1 gene and AHC has been well documented[3]. Although various new pathogenic mutations have been identified over the years, the relationship between mutations in the NR0B1 gene and clinical signs and symptoms is complicated, which may delay timely diagnosis and treatment[4, 5].

Here, we reveal a novel mutation in the NR0B1 gene [c.604delT; p.(C202Afs*62)] in a Chinese family with two patients having different clinical features at onset. The patients inherited the same mutation from their respective mothers, who were heterozygous for the mutation without displaying related clinical features. Recognition of the mutation is of practical importance

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because it shows a genetic pattern of transmission, providing the possibility of finding new cases, even in oligosymptomatic individuals.

1 PATIENTS AND METHODS

Reports describing the case have been performed in accordance with the Declaration of Helsinki and have been approved by the Medical Ethics Committee of Tongji Hospital. Written Informed consent to participate in the study was obtained from participants.

Patient A was a male (age 23 years) who was diagnosed as AHC at 1 year of age, with vomiting, diarrhoea, and convulsions. He was subsequently asymptomatic after treatment with hydrocortisone (10 mg, administered daily, 8 am. to 8 pm.). He was referred to our department with the main complaint of delayed puberty at 14 years of age. He was in a prepubertal stage with testis volume of 2 mL, sparse pubic hair, small penis, and mild and diffuse skin pigmentation (table 1). His bone age (14 and 18 years) was significantly lower than his actual age (17 and 23 years), respectively (fig. 1). After he was diagnosed as HH, including a small penis (3 cm) and low testicular volume (3 mL bilaterally) (Tanner stage 1), he was treated with human chorionic gonadotropin (hCG) and human menopausal gonadotropin (hMG) for 6 years. Currently, at age 23, he is 183 cm tall, and presents with a testicular volume of 8 mL and a small penis. Although his testicular volume increased after treatment, the patient continues to have no sperm production.

Patient B was a baby (2 months of age) with a familial relationship to patient A (fig. 2). He was born after an uneventful pregnancy. On the third day after birth, the patient began to present clinical symptoms

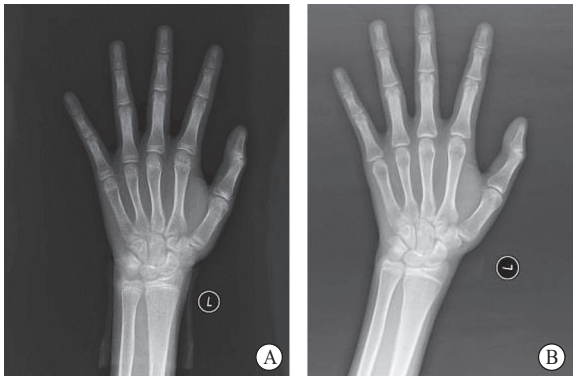

Fig. 1 Bone ages of the patient A at 17 years and 23 years were 14 years (A) and 18 years (B), respectively

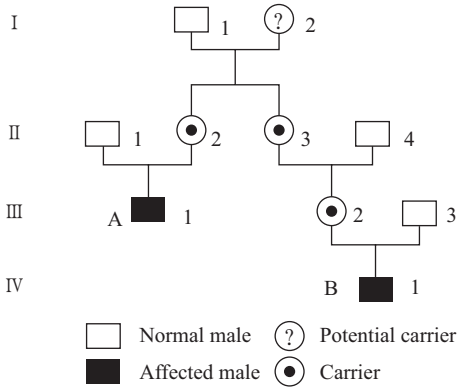

Fig. 2 Pedigree of the family
Horizontal bars above the symbols represent availability for genetic testing.

of AHC including salt loss, vomiting, a prolonged period of postnatal jaundice, skin hyperpigmentation, hyponatraemia, and hyperkalaemia (table 1). Laboratory tests revealed a high serum ACTH concentration (1188 pg/mL; normal range ≤100 pg/mL) and normal serum cortisol concentration (285.57 nmol/L; normal range, 135–650 nmol/L). The patient has been treated with hydrocortisone since that time. His current serum concentrations of ACTH (<5 pg/mL) and 17-hydroxyprogesterone (17-OHP) (0.24 ng/mL) were below the normal range; however, his clinical symptoms have been alleviated markedly.

Both patients were born to non-consanguineous parents and, although no other known cases of X-linked AHC and HH were present in the family pedigree (fig. 2), their respective mothers and common grandmother were identified as carriers. The family members, with the exception of the patients, were healthy. They did not exhibit any hormone insufficiency disorders, such as adrenal insufficiency.

Blood samples were collected and frozen at –20°C until subsequent analyses. Biochemical measurements were performed in the Molecular Endocrinology Laboratory of the Department of Paediatric Endocrinology at Tongji Hospital. Genetic analyses of the NR0B1 gene were performed by Beijing Mygenostics Co., Ltd. (China), and the sequences were compared to the Human Gene Mutation Database (www.hgmd.org) (table 2).

2 RESULTS AND DISCUSSION

X-linked AHC is a rare developmental disorder

Table 1 Age at diagnosis, mode of presentation, puberty (if already reached) of all patients

Case	Age	Age and symptoms at diagnosis	Mode of presentation
Patient A	23 years	1 year, vomiting, hypoglycemia	Delayed puberty, sparse pubic hair, small penis, cryptorchidism and infertility
Patient B	2 months	3 days, salt wasting, vomiting, postnatal jaundice, skin hyperpigmentation	

Table 2 Primers used for amplification and mutagenesis of NR0B1

Primer	Sequence (5′→3′)	Size (bp)
NR0B1-exon1 F	CCACCTGTGGACTCTTGAGC	248
NR0B1-exon1 R	GTGGGACCGCTCCTACTTC	
β-actin-F	GCCGGGACCTGACTGACTAC	100
β-actin-R	TTCTCCTTAATGTCACGCACGAT	

of the human adrenal cortex caused by NR0B1 gene mutations or deletions in the coding region, which predominantly affects males[1]. The NR0B1 gene, composed of two exons separated by a single intron, encodes an unusual 470-amino acid orphan nuclear receptor that is structurally related to the ligand-binding domain localized in the carboxyl terminus of other nuclear receptors, but lacks the typical zinc finger DNA-binding domain in the amino terminus (fig. 2)[5]. More than 200 different mutations have been described in the three segments (two exons and the ligand binding domain) of the NR0B1 gene, most of which are nonsense or frameshift mutations that cause premature truncation of the protein[3].

In this retrospective study, we present two male patients with a novel NR0B1 gene deletion that disrupts the entire coding sequence of NR0B1. Direct sequencing of all coding exons of the NR0B1 gene and their flanking intronic sequences demonstrated that the probands had an identical mutation in exon 1, comprising a single thymine deletion at position 604; this nucleotide deletion resulted in a frameshift and premature stop codon at position 202 [c.604delT; p.(C202Afs*62)] (fig. 3 and 4). Another frameshift mutation (c.605delG) of the NR0B1 gene was reported previously in a patient with AHC and HH[6]. Further analyses of the family members showed that their mothers and grandmother were heterozygous carriers (fig. 2). By comparison with the Human Gene Mutation Database, this mutation in the NR0B1 gene was a novel mutation associated with AHC and HH.

X-linked AHC is predominantly caused by point mutations in the NR0B1 gene; however, it may account for either classic AHC or a phenotype with additional features at various stages of life, resulting from its partial or complete deletion. Genotype-phenotype correlation is therefore uncertain in patients with X-linked AHC. NR0B1 is a transcription factor expressed in the adrenal gland, gonads, ventromedial hypothalamus (VMH), and pituitary gonadotropic cells. Most patients with X-linked AHC present with adrenal insufficiency early in life. The typical clinical manifestations of adrenal insufficiency include feeding difficulty, vomiting, dehydration, hyponatraemia, and hyperkalaemia; however, symptoms vary and may even differ among patients with the same mutation[2]. Previous studies have corroborated such phenotypic heterogeneity associated with NR0B1 mutations[3]. Moreover, some families have shown no genotype-phenotype correlations (table 3)[5, 7–10]. Interestingly, while our two patients carried the same mutation, we observed several differences in their clinical features, such as the age of onset. The extracellular matrix and hormones modulate NR0B1 localisation in the human foetal adrenal glands, thus affecting NR0B1 function. Our results suggest that NR0B1 may have tissue-specific functions[11].

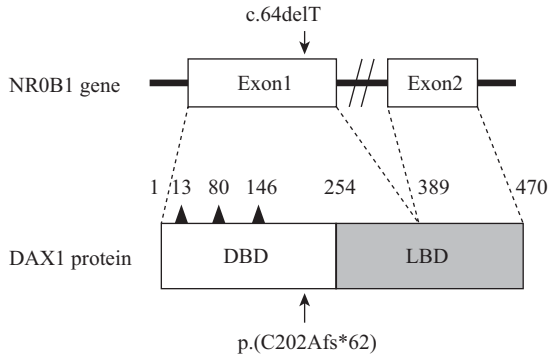

Fig. 3 Schematic representation of the NR0B1 gene and the DAX1 protein[5]
DBD: DNA-binding domain; LBD: putative ligand-binding domain

AHC is characterised by puberty failure and HH in addition to growth hormone deficiency later in life[12]. Delayed puberty due to adrenal insufficiency is extremely uncommon. In the older patient reported here (patient A), puberty was delayed at 14 years without male characteristics, including a lack of penis growth, small testicles, and sparse pubic hair. NR0B1 has a crucial role in the development and function of the reproductive axis at multiple levels. NR0B1 increases gonadotropin releasing hormone (GnRH) expression in the presence of steroidogenic factor 1 in a dose-dependent manner, whereas mutated NR0B1 does not[9]. Thus, HH in AHC may be caused by GnRH downregulation attributable to NR0B1 mutation. Studies in an NR0B1-deficient mouse model have provided evidence that NR0B1 is necessary for proper testicular development and function, which suggests a role beyond that of being simply an "anti-testis" factor[13]. NR0B1 is also expressed in Sertoli cells, and studies have found that male Ahch (DAX1) knockout mice exhibit disordered spermatogenesis and infertility[14]. The patients in our study had low levels of testosterone, follicle stimulating hormone,

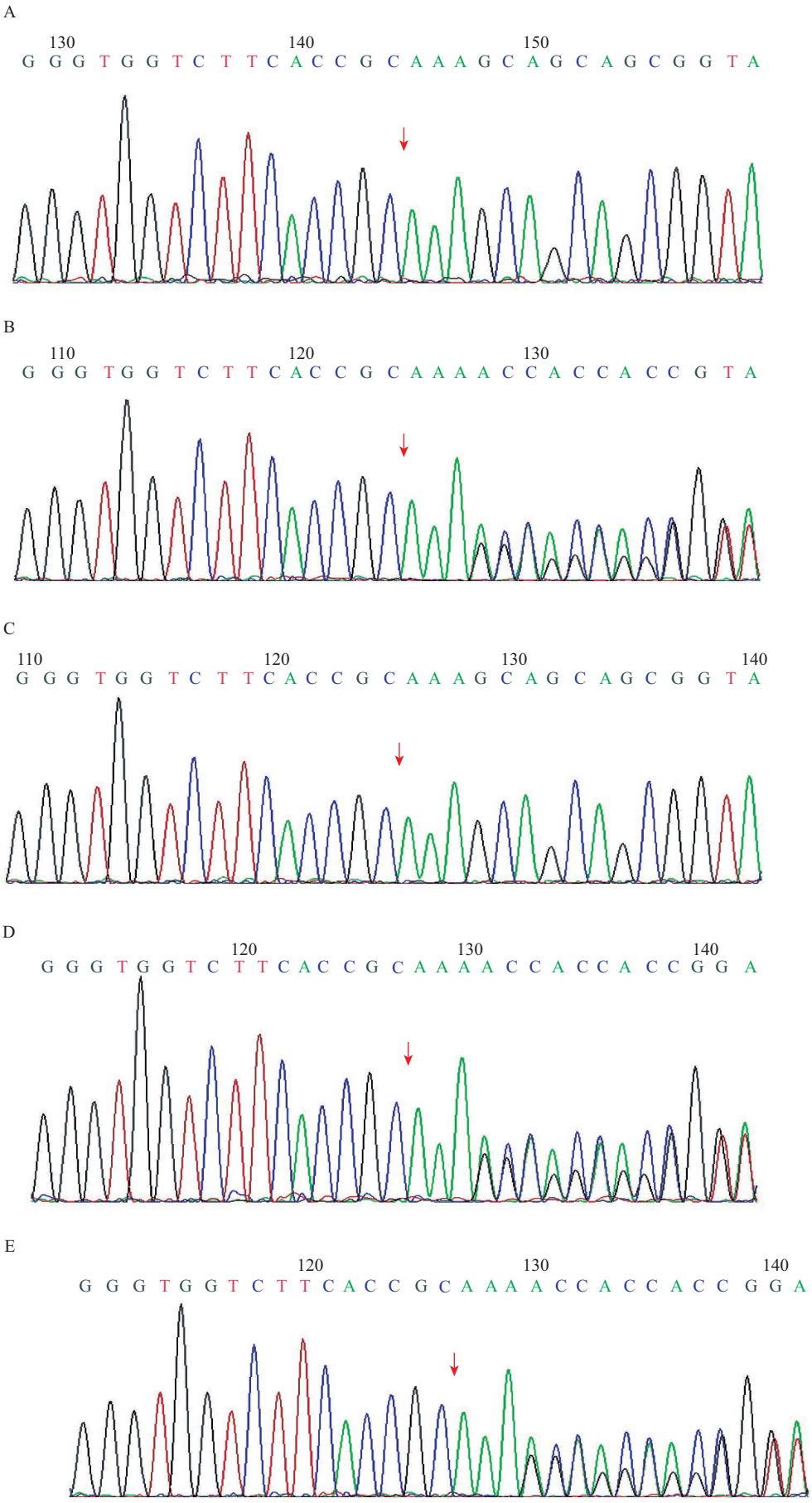

Fig. 4 Sequence analysis of NR0B1 gene mutation in the family
A: patient A; B: patient A's mother; C: patient B; D: patient B's mother; E: patient B's grandmother

Table 3 Age at diagnosis, mode of presentation, puberty (if already reached), and molecular analysis result of all patients in some papers

Case	Age	Age at onset	Extent of the lesion	HH	Mutation	References
1	17.2 yr	7 M	Salt wasting, vomits, hyperpigmentation	Yes	p.Trp39*	[5]
2	30 yr	10 yr	Adrenal insufficiency	Yes	p.Trp39*	[5]
3	17.3 yr	8.5 yr	Salt wasting, hyperpigmentation, oligospermia	Yes	p.Trp39*	[5]
4	16.5 yr	13 yr	Adrenal insufficiency, asthenia, hyperpigmentation	Yes	p.Trp39*	[5]
5	-	21 days	Salt wasting, crisis, fever, fatigue, poor, feeding	-	P353LfsX387	[7]
6	-	21 days	Poor feeding, dehydration, hyperpigmentation	-	P353LfsX387	[7]
7	-	3 days	Poor feeding	-	P353LfsX387	[7]
8	-	8 days	Poor feeding, recurrent vomiting	-	P353LfsX387	[7]
9	18 yr	8 yr	Vomiting, fatigue, and hyperpigmentation	Yes	L262P	[8]
10	10 yr and 9 months	At birth	Hyperpigmentation, repeated vomiting, fever, fatigue, and poor feeding	Yes	L262P	[8]
11	23 yr	8 yr	Fatigue, anorexia, nausea, vomiting, and progressive hyperpigmentation	Yes	C368F	[8]
12	27 yr	At birth	Hyperpigmentation, poor feeding, and Apgar score of 0	Yes	637delC; codon 213 (stop codon 263)	[8]
13	6 M	At birth	Poor feeding, jaundice, progressive hyperpigmentation, and Apgar score of 0	Undetermined	652_653delAC; condon 218 (stop codon 297)	[8]
14	20 yr	10 yr	Progressive fatigue, and hyperpigmentation	Yes	973delC; codon325 (stop codon 371)	[8]
15	15 yr	2 yr	Anorexia, fatigue, and hyperpigmentation	Poor development of secondary characteristic	774_775insCC; codon 259 (stop codon 264)	[8]
16	17 yr	5 yr	Repeated nausea, vomiting, and abdominal pain	Yes	L278P	[8]
17	26 yr	11 yr	Dizziness, fatigue, and hyperpigmentation	Yes	Q222X	[8]
18	-	8 yr	Fatigue, hyperpigmentation	Yes	G329E w/ frameshift	[9]
19	-	At birth	Poor feeding, death at 5th week, hydrocephalus, vacuolated adrenals	Undetermined	Not tested	[9]
20	-	5 weeks	Vomiting, diarrhea, hyponatremia, hyperkalemia	Undetermined	G329E w/ frameshift	[9]
21	-	At birth	Hypothermia, hypogycemia, hyponatremia, hyperkalemia	Undetermined	G329E w/ frameshift	[9]
22	-	2 weeks	Dehydration, weight loss, hyponatremia, hyperkalemia	Undetermined	G329E w/ frameshift	[9]
23	15 yr	0.17 yr	Poor feeding, vomiting, pigmentation	Yes	G183V,263X	[10]
24	20 yr	3 yr	Pigmentation, vomiting, dehydration, hypotension	Yes	L386P	[10]
25	16 yr	5 yr	Pigmentation, hypotension	Yes	L386P	[10]
26	18 yr	5 yr	Pigmentation, hypotension	Yes	Q409X	[10]
27	17 yr	9 yr	Pigmentation, vomiting, dehydration, hypotension	Yes	Ins4281	[10]
28	19 yr	5 yr	Poor feeding, pigmentation	Yes	A292R,371X	[10]
29	21 yr	4 yr	Pigmentation, vomiting, high fever, hypotension	Yes	G136R,137X	[10]
30	15 yr	4 yr	Pigmentation, vomiting,	Yes	W235X	[10]
31	34 yr	5 yr	Pigmentation, vomiting, high fever, coma, diarrhea, hypotension	Yes	Q276X	[10]

Yr: years; M: months; HH: hypogonadotropic hypogonadism

and luteinizing hormone, supporting this theory. Several studies have indicated that treatment with hCG is ineffective for treating ACH and HH in patients. However, when the proband (patient A) was treated with hCG and hMG, his testosterone level eventually reached the range of a normal male; that said, his testicular size increase was below the range of a normal male, and he continued to show no spermatogenesis. Many studies have shown that the majority of patients with AHC have HH and reproductive dysfunction in adulthood (table 3)[5, 7–10]; although HH can be effectively improved by drug intervention, few of these patients are fertile[15]. Therefore, it is possible that the two patients in this study will not achieve spermatogenesis in adulthood.

This study highlights the value of NR0B1 genetic analyses in confirming an AHC diagnosis, and emphasises the critical role of genetic counselling in families of patients with AHC, particularly those with a significant deletion of X chromosome material, including the NR0B1 gene. Our two patients showed clinical features that differed at onset, despite carrying the same mutation. However, it is possible that patient B may exhibit clinical features in adulthood that are similar to those of patient A, such as HH and infertility.

Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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