Late onset X-linked adrenal hypoplasia congenita with hypogonadotropic hypogonadism due to a novel 4-bp deletion in exon 2 of NR0B1

Abstract: We report a novel NR0B1 mutation in a patient affected with X-linked adrenal hypoplasia congenita (X-AHC). The proband first presented with a generalized convulsion at 11 years, 4 months. His clinical and biochemical presentations were consistent with adrenal insufficiency. His basal 17-hydroxyprogesterone (17-OHP) level was not high, and the poor response in 17-OHP on ACTH stimulation test excluded congenital adrenal hyperplasia. At 14 years of age, he did not show any signs of puberty, with low levels of LH, FSH, and testosterone and unresponsiveness to luteinizing hormone releasing hormone stimulation test. Direct DNA sequencing revealed that the proband is hemizygous for a novel NR0B1 mutation (c.1177_1180delGGCC, p.Gly393Cysfs*4). The mother is the conductor of the mutation, which is likely pathogenic as the C-terminus truncated protein lacks the activation function-2 (AF2-TA) transactivation domain, which is highly conserved among members of the nuclear receptor superfamily.

Keywords: adrenal crisis; adrenal hypoplasia congenita; DAX-1; hypogonadotropic hypogonadism; NR0B1; X-linked disease.

Introduction

First reported in 1948, adrenal hypoplasia congenita (AHC) is a rare disorder of adrenal gland developmental failure with an estimated incidence of 1:12,500 (1). It was not until 1994 that the defective gene for X-linked form of AHC (X-AHC, OMIM 300200) was identified, and this was due to the mutation or deletion of nuclear receptor B1 (NR0B1) of chromosome Xp21.3 (2, 3). The classical manifestations of X-AHC are adrenal insufficiency and impairment of puberty. The adrenal gland developmental failure results in the absence of the permanent adult cortical zone; as a consequence, salt-wasting crisis is a typical feature that appears during the first 2 months of life in the majority of the cases (4,5). Absence or arrested puberty with hypogonadotropic hypogonadism (HHG) is usually recognized during adolescence, which is thought to arise from the combined defect of hypothalamic and pituitary function (6).

NR0B1 spans 5.5 kb genomic sequence on chromosome Xp21.3 within the dosage sensitive sex reversal (DSS) locus (DSS, AHC chromosome X critical region, gene 1). Thus, the encoded protein is called “DAX-1”, which has 470 residues. DAX-1 is classified as a member of the nuclear receptor superfamily (NRSF) due the similarity of its C-terminal sequence to the conserved ligand binding domain (LBD) of nuclear receptors (2). DAX-1 is considered an orphan nuclear receptor because its cognate ligand has not yet been identified. The DAX-1 protein does not have the canonical “domain-C” as found in nuclear receptors, which contain the two-zinc finger modules that form the conserved DNA binding domain (7). Instead, the N-terminal sequence of DAX-1 (residues 1-253) contains 3.5 repeating units of 65–67 amino acids with three LXXLL sequence motifs. The LXXLL motif has been originally recognized as a common feature in nuclear co-activators, which is essential for their interaction with nuclear receptors (8). DAX-1 is expressed in the affected tissue and organs linked to X-AHC, which include the hypothalamus, pituitary, adrenal cortex, and gonadal tissues (9). In this report, we present the clinical and genetic evidence to support the diagnosis of a case of late onset X-AHC with HHG in a Malaysian patient.

Materials and methods

Case presentation

The patient is a boy of Malay ethnicity from a family of non-consanguineous marriage. He first presented with a generalized convulsion...
associated with high fever and poor oral intake at 11 years, 4 months. Examination revealed a small boy, with a height of 132 cm (–1.90 SDS) and weight of 26 kg (–2.25 SDS). Biochemical tests revealed the following serum profile: low Na⁺, 129 mmol/L (normal range: 135–145); normal K⁺, 4.2 mmol/L (normal range: 3.5–4.5); low Cl⁻, 98 mmol/L (normal range: 100–108); high ACTH, 931 pg/mL (normal range: 0–46); low cortisol, 122 nmol/L (normal range: 140–700); high renin, 1661 μU/mL (normal range: 5–47); normal 17-OHP (1.1 nmol/L); and undetectable DHEAS (<0.4 μmol/L, normal 2.2–15.2). Short synacthen test (250 μg tetracosactrin) showed neither elevation of cortisol nor of 17-OHP. His genitalia was normal, but gonads were not palpable. Hydrocortisone (10 mg/m²/day) and fludrocortisone (150 μg/day) were started. Hydrocortisone dosage was later increased to 15 mg/m²/day as ACTH levels were markedly increased by up to 1250 pg/mL. At the age of 14 years, his testes were 2 mL bilaterally, pubic hair at Tanner Stage 1, and with low levels of serum LH (<0.1 mU/mL), FSH (0.9 mU/mL), and testosterone (0.4 nmol/L). He showed no response to lutenizing hormone-releasing hormone (LHRH) stimulation test, suggesting hypogonadotrophic hypogonadism. Testosterone enanthate replacement therapy was then initiated.

Apart from AHC, he also had a small, doubly committed subarterial ventricular septal defect (VSD), which did not compromise his health and daily activity. At the age of 16 years, he had a thoracotomy for closure of VSD without any complication. He had no further admission for adrenal crisis. His blood pressure was always in the normal range, but he continued to be markedly pigmented – he admitted that he was not compliant to hydrocortisone. At the age of 19 years 10 months, his basal serum LH and FSH were 0.1 mU/mL, respectively. At the age of 20 years, his hydrocortisone was replaced with twice daily prednisolone at 5 mg/m²/day to improve his compliance, along with 100 μg/day fludrocortisone. His ACTH levels improved to <25 pg/mL. On follow up 6 months later, he was noticeably not as pigmented as before and showed no cushingoid features. His height was 171.2 cm (–0.79 SDS, MPH –1.51 SDS), and his weight was 60.3 kg (BMI 20.7 kg/m²). His testes were 3 mL bilaterally, and he continued receiving 250 mg intramuscular testosterone enanthate every 2 weeks. He is currently attending college and has no recent complaints of lethargy.

**NR0B1 mutational analysis**

After obtaining written informed consent, blood DNA was isolated from the parents and the proband using standard methods. Primers were designed to include approximately 50-bp **NR0B1** intron-exon junctions (exons 1, 2 and 2A) for amplification by polymerase chain reaction (PCR; primers and conditions are available on request). PCR products were purified and directly sequenced.

**Results**

The proband was found to be hemizygous for a 4-bp deletion [c.1177_1180delGGCC] in exon 2 of **NR0B1** (Figure 1A). This mutation is considered a novel one because it is not listed in the most recent version of Human Genome Mutation Database Professional 2013.2. The 4-bp deletion is a frameshifting mutation that resulted in the formation of a premature stop codon (TGA), three codons downstream of the mutation site. The mutation did not create a novel restriction enzyme site, but disrupted an *HpaII* site in the wild-type **NR0B1** sequence. *HpaII* PCR-RFLP (Figure 1B) and Sanger sequencing indicated that the mother is heterozygous for the mutation, and the father does not have the mutant allele.

**Discussion**

Since 1994, the identification and characterization of DAX1 as the underlying cause of X-AHC has had significant impact on the understanding and management of this complex disorder.
implications for diagnosis of individuals and families with this condition. There are currently 205 NR0B1 mutations listed in Human Genome Mutation Database; of these, 199 are associated with HHG, adrenal insufficiency, and adrenal hypoplasia. The proband was initially suspected to have the more common disorder of congenital adrenal hyperplasia. However, his serum cortisol and 17-OHP levels were not elevated on ACTH stimulation test, which suggested X-AHC. Hypogonadotropic hypogonadism, another common feature of this disorder, usually becomes apparent during adolescence, with impaired or arrested pubertal development leading to poor height gain. Given that the proband did not show any signs of pubertal development at 14 years, his puberty was induced and long term sex steroids replacement therapy were continued. The proband serum FSH and LH levels were still low when he was 19 years 10 months, thus suggesting hypogonadotropic hypogonadism. In our opinion, the VSD condition in the proband did not compromise his growth and development. This is because he showed a good catch-up growth, from −2.03 SDS at the time of initial testosterone administration, to the current final height of −0.79 SDS, which is far better than the expected mid-parental height at −1.51 SDS.

X-AHC has been proposed to arise from defective DAX-1 nuclear localization. This is because functional assay has revealed that DAX-1 mutant proteins have impaired nuclear localization, thus interfering with its transcriptional repression activity (10). The LXXLL motifs in the N-terminal region and the AF2 domain within the C-terminus region have also been shown to have an essential role in DAX-1 nuclear localization. The majority of DAX-1 mutations reported to date also clustered within the C-terminus, highlighting the importance of the LBD-like structure (5). The 4-bp NR0B1 deletion in the proband is a frameshifting mutation, which forms a premature stop codon at position 396 (p.Gly393Cysfs*4). The mutation is predicted to exclude 77-amino acids from the C-terminus region of DAX-1. This resulted in the loss of the putative helix-12 in the LBD-like structure containing the AF2 domain (Figure 1C), which has been shown to have a crucial part in DAX-1 nuclear localization and transcriptional repression activity (5, 11, 12); thus, the truncating mutation in the proband is likely to be very disruptive.

The proband had a late elder brother who was most likely affected with X-AHC because his mother described him as having pigmented skin and died at the age of 10 years, after a week of high fever and poor oral intake. This could be attributed to an undiagnosed Addisonian crisis, although no definitive diagnosis of his death was available. Aside from this, there was no other history of similar presentation in immediate family members, especially among the maternal male relatives.

It is conceivable that X-AHC disease presentation would typically appear during early infancy, given that the disorder resulted in adrenal gland developmental failure. This explains why the majority of the cases presented the disease during the first 2 months of life. However, despite the disruptive mutation in the proband, it is unknown why (and his late elder brother) appear to have manifested the disease during childhood. Childhood and adult onset X-AHC have been reported in a number of X-AHC cases (5).

The current literature has indicated that the type or location of DAX1 mutation does not consistently predict the age of disease presentation or disease severity; furthermore, a direct genotype-phenotype correlation does not exist. For example, a severely truncating mutation p.W171X has been reported to display different ages of disease presentation in three families; the first family has a member who presented at the age of 7 years, while two affected male siblings from another family presented during the neonatal period (4). Another family with two affected male siblings presented at the age of 3 and 6 years, respectively (13). Calliari et al. described three male siblings with the same DAX-1 mutation (p.Q359X); of these, two presented during the neonatal period, while the eldest brother presented at the age of 6 years (14).

Thus, X-AHC patients could present heterogeneous phenotypes, in which the age of onset of adrenal insufficiency, disease severity and manifestation could vary, even among family members with the same mutation. Additionally, although X-AHC is classically associated with impairment of puberty, there have been recent reports of DAX1 mutations associated with pubertal variants, including normal puberty, gonadotropin-independent precocious puberty, and central precocious puberty (5, 15, 16). A unifying picture to explain the heterogeneous disease presentation remains elusive at the present time, and may require an understanding of the DAX1 spatiotemporal mode of action and the network partner(s) involved. However, it is possible that genetic, epigenetic, and non-genetic factors could also influence the disease outcome.

In summary, we identified a novel 4-bp deletion in exon 2 of NR0B1, which causes childhood onset X-AHC with HHG. This expands the spectrum of pathogenic mutations that cause X-AHC and re-emphasizes the functional importance of DAX-1 LBD-like structure in the disease pathogenesis.
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References