NOVEL *DAX-1* (*NR0B1*) MUTATION IN A THAI BOY WITH X-LINKED ADRENAL HYPOPLASIA CONGENITA (AHC): A FIRST REPORT

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ABSTRACT

Background: Adrenal hypoplasia congenita (AHC) is a rare inherited disorder of adrenal development resulting in hypoplasia of the adrenal gland and inability to produce glucocorticoids, mineralocorticoids and sex steroids. X-linked AHC is the most common form and is caused by mutation of the *DAX1* gene. Here, we report a case of a 4-year-old Thai boy with X-linked AHC, the first case of genetically confirmed novel *DAX1* mutation in Thailand.

Patient and Method: A 4-year-old Thai boy presented with fever and vomiting for 3 days. He was the 5th child of unrelated parents. The past medical history was unremarkable except his skin had been progressively more pigmented since early infancy. Physical examination revealed normal genital development and addisonian hyperpigmentation, prominent at the skin crease, gums and knuckles. Laboratory investigations showed hyponatremia, hyperkalemia and hypochloremia. Hormonal evaluation revealed low baseline cortisol level without rising after 250 ug ACTH stimulation test. ACTH level and plasma renin activity were elevated. A diagnosis of X-linked AHC was established. Glucocorticoid and mineralocorticoid replacement therapy were initiated. Molecular analysis by direct DNA sequencing of the *DAX1* gene was performed among patient and family members.

Result: Mutation analysis revealed a novel hemizygous GG deletion (c.1148_1149delGG) resulting in a premature termination codon at position 387 (p.Gly383Aspfs*5). This frameshift mutation was predicted to encode a truncated *DAX1* protein missing a portion of ligand binding domain. Molecular analysis from other family members revealed that the mutation was inherited from the carrier mother.

Conclusion: We report a case of classical X-linked AHC establishing a novel frameshift mutation in the *DAX1* gene. Genetic analysis is important not only to confirm diagnosis in the index case but also to detect the carrier status in his mother, providing proper management and appropriate genetic counseling for this family.

Keywords : Mutation analysis, *DAX1* gene, X-linked adrenal hypoplasia congenita, Thailand

J Southeast Asian Med Res 2017; 1: 81-84.
http://www.jseamed.org

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Introduction

Adrenal hypoplasia congenita (AHC) is a rare inherited disorder of adrenal development resulting in hypoplasia of the adrenal gland and inability to produce glucocorticoids, mineralocorticoids and sex steroids.[1] The most prevalent form is an X-linked AHC (AHC; OMIM: 300200) caused by mutation of the *NR0B1* (nuclear receptor subfamily 0, group B, member1) or *DAX1* (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1; OMIM: 300473) gene. While AHC has a prevalence of 1:12,500 live births, X-linked AHC has an estimated prevalence between 1:140,000 and 1:1,200,000 children.[2] Most patients present skin hyperpigmentation and salt losing crisis.

The *DAX1* gene is located at Xp21.3 and consists of two exons.[3] This gene encodes a 470 amino acid *DAX1* protein belonging to the orphan member of the nuclear receptor superfamily.[3] *DAX1* is expressed in the adrenal cortex, ventromedial nucleus of the hypothalamus, gonadotropes of the anterior pituitary gland, Sertoli and Leydig cells in the testis and theca and granulosa cells in the ovary. *DAX1* nuclear receptor acts as a transcriptional repressor of genes involved in normal gonadal and adrenal development and regulates the hypothalamus-pituitary-gonadal axis.[3,4] To date, more than 100 mutations in the *DAX1* gene have been identified but no data was available in Thailand.[5]

Here, we report the clinical and molecular analysis of a 4-year-old Thai boy with classical X-linked AHC, which identified a novel frameshift mutation in the *DAX1* gene. Our patient was the first case of genetically confirmed *DAX1* mutation in Thailand.

Patients and Methods

Patients

A 4-year-old Thai boy attending our hospital presented fever and vomiting for three days. He was the fifth child of nonconsanguineous parents and was born at term with uneventful pregnancy and delivery. Birth weight was 3,200 gm. He has two older brothers (33 and 29 year) and two older sisters (30 and 20 year). All are healthy. The past medical history was unremarkable except his skin became progressively more pigmented since early infancy. No history existed of unexplained death of male infants or children in the family. Physical examination revealed normal vital signs, no dysmorphic feature with normal genital development and addisonian hyperpigmentation, prominent at the skin crease, gums and knuckles. Laboratory investigations showed hyponatremia (Na 128.4 mmol/L), hyperkalemia (K 5.2 mmol/L) and hypochloremia (Cl 94.4 mmol/L). Hormonal evaluation revealed low baseline cortisol level (0.89 ug/dL) without rising after 250 ug ACTH stimulation test. ACTH level was very high (13,270 pg/mL, N 0-71) as well as plasma renin activity (470 ng/dl/hr, N 20-280). Aldosterone and 17-hydroxyprogesterone levels were normal. The karyotype was 46, XY. Computerized tomography (CT scan) showed normal size of both adrenal glands. After diagnosis of X-linked AHC was established, glucocorticoid and mineralocorticoid replacement therapy were initiated.

Mutation analysis of the *DAX1* gene

After institutional review board approval, informed consent was obtained from the patient and family members. Genomic DNA was extracted from peripheral blood lymphocytes using a commercial available kit according to manufacturer protocols. Two coding exons and exon-intron boundaries of the *DAX1* gene were amplified by polymerase chain reaction using four pairs of primers as previously described.[6] The PCR products were purified and directly sequenced in both forward and reverse directions. The reference sequences were NM_000475.4 for *DAX1* cDNA and NP_000466.2 for *DAX1* protein, respectively.

Results

Mutation analysis by direct DNA sequencing of all two coding exons and exon-intron junction of the *DAX1* gene revealed a novel hemizygous GG deletion; c.1148_1149 delGG or p.Gly383Aspfs*5, which is located near the end of exon 1 **(Fig. 1)**. This frameshift mutation resulted in a premature termination codon at the position 387 and was predicted to encode a truncated *DAX1* protein missing normal 87 amino acids of the C-terminal. Alternatively, this mutation resulted in nonfunctional truncated *DAX1* protein missing a part of the ligand binding domain. Molecular analysis of the *DAX1* gene in other family members including

the proband's mother and one of the proband's sisters was also performed and revealed a heterozygous status for the same mutation only in the maternal DNA. Thus, the mutation identified in our patient was inherited from his carrier mother.

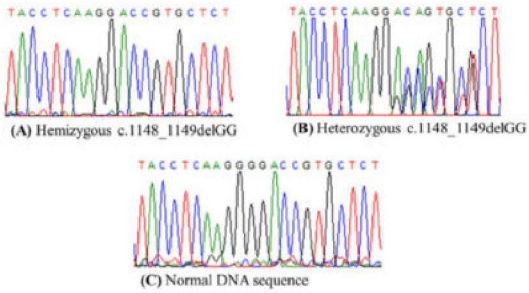

Fig. 1 Electropherogram of the genomic DNA revealed a novel hemizygous frameshift; c.1148_1149delGG, mutation in exon 1 of the *DAX1* gene in a 4-year-old boy affected with X-linked AHC ***(A)***, a heterozygous c.1148_1149delGG mutation in the carrier mother ***(B)*** and a normal DNA sequence in the proband's sister.

Discussion

X-linked AHC is a rare inherited disorder of the adrenal gland.[1] Most patients present signs of primary adrenal insufficiency including hypoglycemia and skin hyperpigmentation and salt-losing crisis (hyponatremia, hyperkalemia and metabolic acidosis), in early infancy or childhood.[2] However, these symptoms might first present in adulthood.[7] Additional features include delayed puberty and infertility resulting from hypogonadotropic hypogonadism, a defect in spermatogenesis and progressive loss of testicular function.[8-11] The diagnosis of X-linked AHC is based on clinical findings and hormonal studies and these must be distinguished from other more common causes of primary adrenal insufficiency especially 21-OHD congenital adrenal hyperplasia. The serum 17-OHP and androgen profiles, high in 21-OHD but low in X-linked AHC, are the key to differential diagnosis for these two conditions. However, the definite diagnosis is genetic analysis for *DAX1* mutation. Glucocorticoid and mineralocorticoid replacement therapy is the cornerstone for treatment. In this study, our patient presented adrenal crisis during illness in early childhood. He had skin hyperpigmentation, hyponatremia and hyperkalemia. His serum ACTH was very high but serum cortisol and 17-OHP levels were not elevated after the ACTH stimulation test. All of these were suggestive of X-linked AHC. After intravenous high dose hydrocortisone and 9-alpha fludrocortisone was given, his clinical signs improved and he was finally discharged home with long term glucocorticoid and mineralocorticoid replacement.

Our patient was too young for evaluated hypogonadotropic hypogonadism. However, he should be followed up until adolescence and testosterone replacement might be necessary to initiate pubertal development.

DAX1 mutation is a relatively frequent (more than 50%) cause of adrenal insufficiency among boys with primary adrenal hypoplasia and almost all cases among boys who have a family history compatible with an X-linked inheritance pattern.[3,12] To date, more than 100 different *DAX1* mutations have been identified including large gene deletions, missense, nonsense and frameshift mutations.[2,5,13-17] Most of the mutations are either nonsense or frameshift mutations causing premature truncation of *DAX1* protein. These truncated mutations are located throughout the *DAX1* gene; whereas, most of the missense mutations are clustered in highly conserved amino acids in the ligand-like binding domain.[2, 5, 13-17] As in our patient, mutation analysis identified a novel hemizygous frameshift mutation; c.1148_1149delGG or p.Gly383Aspfs*5, in exon 1 of the *DAX1* gene. This mutation is predicted to result in a premature termination at the codon 387 causing a truncation of the carboxyl terminus or missing a portion of the ligand binding domain, which plays an important role in functional *DAX1* protein. To our knowledge, our patient was the first case of genetically confirmed *DAX1* mutation in Thailand.

Once our index case has been identified, familial investigation must be required due to possible X-linked transmission. Unfortunately, we received the blood samples only from the proband's mother and one of the proband's sister. Molecular analysis of the *DAX1* gene was performed revealing a heterozygous status for the same mutation only in the maternal DNA, indicating that our proband's mutation was inherited from his carrier mother. Genetic analysis of *DAX1* is important not only to confirm diagnosis in the proband, but also to detect the carrier status in other family members, providing proper management and appropriate genetic counseling to the family members. Moreover, this genetic information is also helpful for prenatal diagnosis or pre-implantation genetic diagnosis for family members in the future.

Conclusion

In conclusion, we reported a 4-year-old boy presenting classical X-linked adrenal hypoplasia congenita (AHC). Mutation analysis revealed a novel frameshift mutation of the *DAX1* gene, which was inherited from a heterozygous mother. In addition to establishing the correct diagnosis, molecular analysis of the *DAX1* gene was also helpful in carrier detection leading to appropriate genetic counseling and proper management for this family.

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