X-Linked Adrenal Hypoplasia Congenita and Hypogonadotropic Hypogonadism: Mutation of the DAX-1 Gene in a Patient

DAX-1 Gen Mutasyonu Saptanan Bir Olgu: X-Geçişli Konjenital Adrenal Hipoplazi ve Hipogonadotropik Hipogonadizm

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ABSTRACT X-linked adrenal hypoplasia congenita (AHC), an inherited disorder of the development of the adrenal cortex which results from the loss of function mutations of the DAX1 gene is frequently associated with hypogonadotrophic hypogonadism. Here, we report a case of a 2 month-old boy who initially presented with salt-losing primary adrenal failure and at follow-up diagnosed as hypogonadotropic hypogonadism. Genetic analyses of the patients was found a mutation at the C-terminus of exon 1 of the DAX-1 gene, which is a 1-base deletion (423DelG) inherited from the mother. In this case, close patient follow-up and genetic confirmation of the disease led to prompt identification of the patient's gonadal axis deficiency and this minimized the deleterious consequences of an erroneous diagnosis. We present this rare cause of primary adrenal failure in infancy in order to highlight the importance of the early precise diagnosis of patients with AHC.

Key Words: NR0B1 protein, human; X-linked adrenal hypoplasia congenita; hypogonadism

ÖZET X-geçişli konjenital adrenal hipoplazi (KAH) DAX-1 genin fonksiyon kaybedici mutasyonu sonucu gelişen, sıklıkla hipogonadotropik hipogonadizm ile ilişkili adrenal korteksin kalıtımsal bir bozukluğudur. Bu olgu raporunda, tuz kaybı ile giden primer adrenal yetersizlik ve izlemde hipogonadotropik hipogonadizm tanısı alan 2 aylık erkek olgu sunulmuştur. Hastanın genetik analizinde, anneden kalıtılan DAX-1 genin ekzon-1'in C terminalinde 1-bazlık delesyon (423DelG) saptanmıştır. Bu olguda, hastanın yakın izlemi ve genetik tanısı, gonadal eksen yetersizliğinin zamanında saptanmasını ve yanlış tanının önüne geçilmesini sağlamıştır. Bu olgu raporunda, süt çocukluğu döneminde primer adrenal yetersizliğin nadir bir nedeni olan KAH antitesinin erken tanısının önemini vurgulamak istedik.

Anahtar Kelimeler: NR0B1 proteini, insan; X-geçişli adrenal hipoplazi konjenita; hipogonadizm

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DAX-1 [dosage-sensitive sex reversal, adrenal hypoplasia congenital, X chromosome (Xp21.3-21.2)] (NR0B1) is a 470 amino acid orphan nuclear receptor that plays a key role in the development and function of the adrenal gland and hypothalamic pituitary-gonadal axis. It is expressed in the adrenal gland, gonads, ventromedial hypothalamus and pituitary gonadotrophs.[1] Mutations in *DAX1* result in primary adrenal failure and hypogonadotropic hypogonadism (HH) in human. Majority of *DAX1* mutations are frameshift or nonsense mutations, which cause premature truncation of the protein.[2] Relatively few missense mutations all of which are located within the carboxy-terminal half of the protein are also reported.[3] Until now, more than 80 different mutations in more than 70 individuals or families with X-linked adrenal hypoplasia congenita (AHC) have been reported.[4]

Here, we report a patient with genetically confirmed AHC and hypogonadotropic hypogonadism to emphasize that boys with adrenal cortical hypofunction should be suspected of X-linked AHC and molecular analysis of DAX1 gene should be performed. The follow-up of sexual development of these patients is important for the correct timing of sex steroid replacement.

CASE REPORT

The patient was the first living son of non-consanguineous parents born at term after an in vitro fertilization pregnancy. The delivery and post and perinatal course were uncomplicated. His birth weight was 3250 g. He first presented to our department when he was two months old with the complaints of vomiting, dehydration and failure to thrive. The family history was significant for previously undiagnosed two male siblings with similar clinical features, including fatigue, dehydration and hyperpigmentation followed by death. In addition, three maternal uncles had died within the first two months of life because of failure to thrive, and his maternal aunt had a four-year-old son who had been diagnosed as having adrenal insufficiency in Germany (Figure 1). On physical examination, his weight was 4020 g (-1.28 SDS), with a length of 56 cm (-0.25 SDS). His blood pressure was normal. He was dehydrated, and generalized hyperpigmentation was noted. His testicular volumes were measured as one mL and the length of the penis was 3.5 cm. Laboratory tests showed metabolic acidosis with moderate hyponatremia (Na: 127 mEq/L) and hyperpotassemia (K: 8.9 mEq/L). His blood glucose was normal. His plasma ACTH level was 270 pg/mL (normal: 0-100), plasma cortisol (08:00 am) level was 10.03 μg/dL. The serum 17-OH progesterone level was 0.9 ng/mL (normal: 0.1-0.9), DHEA-S was 55 μg/dL (normal: 17-77), aldosterone was 70 pg/mL (normal: 20-760) and plasma renin activity was 26 ng/mL/h (normal: 2.2-10.2). A standard dose ACTH stimulation test was performed and inadequate response of cortisol (basal cortisol: 9.7 ng/mL, peak cortisol: 14 ng/mL) led to the diagnosis of adrenal insufficiency thus steroid replacement using hydrocortisone and fludrocortisone was initiated.

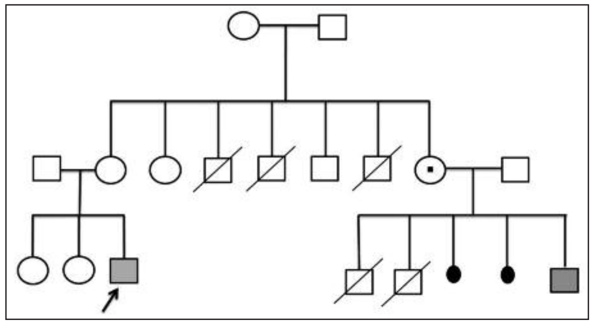

FIGURE 1: Pedigree of the family.

At 14 years of age, his genitalia remained prepubertal (penile length 5 cm and testis volume 3 ml). Inadequate increase of LH and FSH in GnRH stimulation test (Table 1) suggested HH. Therefore testosterone replacement therapy was initiated.

Genetic analysis was conducted after obtaining informed consent from the parents. Genomic DNA was extracted from peripheral blood leukocytes. Both exons of DAX1 gene were amplified by PCR. Genetic analysis revealed a known mutation at the C terminus of exon 1 of the *DAX-1* gene that being a 1-base pair deletion (423DelG) inherited from the mother (Figure 2).

DISCUSSION

X-linked AHC with primary adrenal insufficiency and HH is a rare disorder caused by mutations of *DAX-1*.[2,5] It has an estimated prevalence of 1:12 500 live births.[6] It is predicted that as many as 50% of boys with idiopathic primary adrenal insufficiency may have mutations in DAX1, after other known causes [i.e., congenital adrenal hyperplasia (CAH)] have been excluded.[6] Wider spectrum of clinical presentations have also been reported in patients with *DAX1* mutations.[7] AHC is frequently associated with HH, which is not recognized until the patient reaches adolescence.[6] Boys usually present with salt-losing primary adrenal failure in early infancy (<2 months of life) or rarely in childhood.[2]

TABLE 1: GnRH stimulation test.

Minutes	FSH (mIU/mL)	LH (mIU/mL)	Total Testosteron (ng/mL)
0’	1.65	0.55	< 20
30’	1.46	0.82	
60’	1.53	0.51	
90’	1.46	0.36	
120’	1.47	0.31	< 20

FSH: Follicle stimulating hormone; LH: Luteinizing hormone.

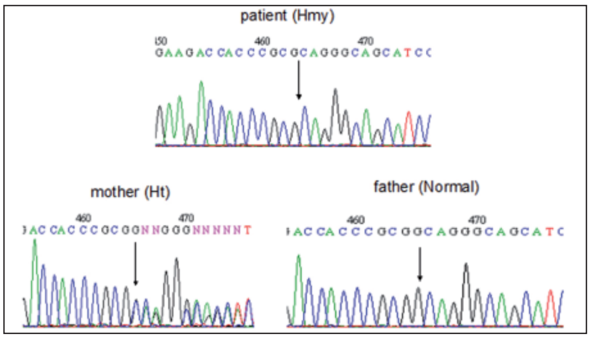

FIGURE 2: Electropherograms showing the DAX1 mutations identified in the proband, mother and father. A black arrow point to the c.423DelG, which results in a frameshift mutation. The electropherograms show a hemizygous mutation in the proband and a heterozygous mutation on the mother. The father does not carry the c.423DelG mutation.

(see color figure at http://www.turkiyeklinikleri.com/journal/pediatri-dergisi/1300-0381/)

Clinical signs and symptoms include typical features of primary adrenal insufficiency: hyperpigmentation, vomiting, poor feeding, failure to thrive, convulsions, vascular collapse and sudden death. Biochemical findings include hyponatremia, hyperkalemia, hypoglycemia, reduced serum cortisol and aldosterone, and increased plasma ACTH.[8] In the current case, the patient presented with vomiting, dehydration, failure to thrive, salt-lose (hyponatremia and hyperpotassemia) and hyperpigmentation due to his primary adrenal failure when he was two months old. He had high ACTH levels despite normal basal cortisol levels, which made us suspicious of adrenal insufficiency. The inadequate response of cortisol in a standard dose ACTH stimulation test led to the diagnosis of adrenal insufficiency. At follow up, DAX 1 mutation was considered due to the accompanying HH. Numerous mutations of the *DAX1* gene, including deletions, alterations of splice sites, missense, nonsense and frame-shift mutations, have been identified to date. The majority of these mutations are frame-shift or nonsense mutations.[6] Missense mutations account for about one-quarter of *DAX1* mutations, while about one-third of AHC patients harbor *DAX1* deletions. Phenotypic heterogeneity occurs within a family with the same mutation, as well as with different *DAX1* mutations, suggesting an influence of modifier genes or environmental effects on the expression of clinical manifestations.[8] A patient with a *DAX1* missense mutation (W105C) had isolated mineralocorticoid deficiency, without evidence of glucocorticoid deficiency or HH.[9] The type or location of a *DAX1* mutation does not always predict disease severity or the age of onset of adrenal insufficiency.[8] In our patient, genetic analysis revealed a 1- base pair deletion at nucleotide position of the *DAX-1* gene (423 DelG) resulting in a frame-shift mutation inherited from the mother. Several single gene disorders have now been shown to cause HH in humans such as *KAL* (anosmin-1) gene, GnRH receptor, pituitary transcription factors (*PROP-1* and *HESX-1*) mutations.[6] SF1 (NR5A1), which is another nuclear receptor structurally related to DAX1, is essential for the development of the HPA and HPG axes. Disruption of the *SF1* gene in mice causes complete agenesis of the gonads and adrenals, as well as hypothalamic and pituitary defect.[8]

In the presence of a *DAX1* mutation, normal function of the gonadal axis has not yet been reported. The defective gonadotropin secretion is thought to be a consequence of disruptions at both the hypothalamic and the pituitary level.[10] DAX-1 plays a role in the central control of puberty. The classically observed puberty disorder in cases with DAX-1 mutations or deletions is hypogonadotropic hypogonadism. In addition, rare cases with gonadotropin-independent precocious puberty have been reported in previous studies. It was hypothesized that chronic excessive ACTH levels may have stimulated Leydig cells, leading to gonadotropin-independent precocious puberty in some boys.[11,12] However, there are many reports of cases whose puberty began spontaneously but later underwent pubertal arrest.[13,14] In the present case, patients' prepubertal genitalia and inadequate increase of LH and FSH in GnRH stimulation test at 14 years of age led to the diagnosis of HH. Through close follow-up, the patient's gonadal axis deficiency was promptly identified, and this enabled an assisted but successful onset of puberty. Loss of function in the hypothalamic-pituitary-gonadal axis over time is the most often result in AHC.[14-16] Infant boys with congenital isolated GnRH deficiency usually have micropenis and cryptorchidism.[17] In many reported AHC cases, despite their proven HH penis sizes of the patients were normal, and the testes were completely descended.[14] Similarly, the patient in this report has presented with normasized penis and completely descended testes. Although the gonadotropin and testosteron levels during mini-puberty were not available for the present case, he might have had progressive disruption in the hypothalamic-pituitary-gonadal axis, which became clear over time. Current treatment approaches for patients with *DAX1* mutations involve maintenance steroid replacement therapy and symptomatic treatment during adrenal crises. To treat HH in affected boys hormonal replacement can be provided at the time of puberty.[18] In the current case, hydrocortisone and fludrocortisone were started at admission and at follow up due to his testosterone replacement was initiated. Some patients with X-linked AHC with deletions in *DAX1* have a contiguous gene syndrome, and present with various combinations of glycerol kinase deficiency, Duchenne muscular dystrophy, ornithine transcarbamylase deficiency and mental retardation, which allowed the responsible gene locus to be narrowed to Xp21.3-p21.2.[8] However, none of the above mentioned diseases have been clinically presented in the current case.

We conclude that determining the precise cause of adrenal insufficiency occurring in infancy is of critical importance for the correct management of affected children. Genetic testing in boys with primary adrenal insufficiency and suspected X linked AHC is of great significance for providing appropriate genetic advice to their families.

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