

Presymptomatic diagnosis of X-linked adrenal hypoplasia congenita by analysis of *DAX1*

John C. Achermann, MRCP, MD, Bernard L. Silverman, MD, Reema L. Habiby, MD, and J. Larry Jameson, MD, PhD

A novel *DAX1* mutation (L381H) was discovered in the asymptomatic 8-month-old brother of a boy with primary adrenal failure. The infant had impaired adrenal reserve despite normal basal adrenal steroid concentrations. This case highlights the value of genetic testing in children at risk of the development of X-linked adrenal hypoplasia congenita before the onset of a potentially life-threatening adrenal crisis. (J Pediatr 2000;137:878-81)

Adrenal hypoplasia congenita is an inherited disorder of adrenal gland development, occurring with an estimated incidence of 1 in 12,500 live births.¹ The X-linked form of this condition is caused by mutations in the *DAX1* gene.^{2,3} *DAX1* encodes an orphan nuclear receptor that is expressed in the hypothalamus, pituitary gonadotropes, gonads, and the adrenal gland.⁴ More than 60 families with >50 different *DAX1* mutations have now been reported.^{5,6} Most of these children have frameshift or nonsense mutations, but 10 missense mutations in *DAX1* have also been found.

Boys with *DAX1* mutations typically have primary adrenal failure during infancy or childhood. The fetal adrenal gland, which normally involutes shortly after birth, contains atypical "cy-

tomegalic" cells.⁷ The adult adrenal gland fails to develop fully. Hypogonadotropic hypogonadism, an integral feature of this condition, usually becomes apparent at the expected time of puberty and has been reported recently in a woman homozygous for a *DAX1* mutation.⁸ However, *DAX1* mutations are likely to be rare in patients with hypogonadotropia in the absence of adrenal failure.⁹

We report the presymptomatic diagnosis of AHC in the younger brother of a boy with a novel missense mutation in *DAX1*. This case highlights the importance of genetic testing to identify individuals at risk of the development of AHC before the onset of an Addisonian crisis and may provide important insight into the role of *DAX1* in human adrenal development.

CASE REPORT: PROBAND

The proband (II:1) demonstrated progressive weight loss and vomiting at the end of the first week of life (Fig 1). He was found to be hyponatremic, and a diagnosis of renal tubular acidosis was made. He remained well throughout infancy but had unusually severe symptoms during episodes of viral illness in early childhood and also had diffuse pigmentation. Prolonged vomiting, hyponatremia, and hyperkalemia after appendectomy at 4 years of age prompted further investigations of adrenal function. These tests confirmed primary adrenal insufficiency, and a diagnosis of X-linked AHC was considered.

AHC Adrenal hypoplasia congenita

METHODS

After consent was obtained, DNA was extracted from blood leukocytes, and *DAX1* was amplified by polymerase chain reaction with the use of primers and conditions reported previously.⁶ The mutation was identified with the primer pair: Forward, 5'-GCCTCAGCGGGCCTGTTGAAG-3'; Reverse, 5'-CCCGATGCTTTTGTGAGCTGGGAA-3'. Direct sequencing was performed with the dRhodamine dye terminator sequencing kit and ABI377 automated sequencer (PE Applied Biosystems, Foster City, CA).

From the Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University Medical School, and Children's Memorial Hospital, Chicago, Illinois.

This work was performed as part of the National Cooperative Program for Infertility Research and was supported by National Institutes of Health grants U54-HD-29164 and PO1 HD-21921. Dr Achermann received support from the Endocrine Fellows Foundation.

Submitted for publication Feb 3, 2000; revision received Apr 13, 2000; accepted May 3, 2000.

Reprint requests: J. Larry Jameson, MD, PhD, Endocrinology, Metabolism, and Molecular Medicine, Northwestern University Medical School, 303 East Chicago Ave, Tarry Building 15-709, Chicago, IL 60611.

Copyright © 2000 by Mosby, Inc.

0022-3476/2000/\$12.00 + 0 9/22/108567

doi:10.1067/mpd.2000.108567

RESULTS

Direct DNA sequencing of *DAX1* revealed a novel L381H (CTC > CAC) missense mutation in the proband (Fig 1, II:1). This amino acid is located within the putative ligand-binding domain of this nuclear receptor. The patient's mother was heterozygous for this mutation. Screening of his younger brother revealed an identical hemizygous L381H missense mutation in *DAX1* (Fig 1, II:2).

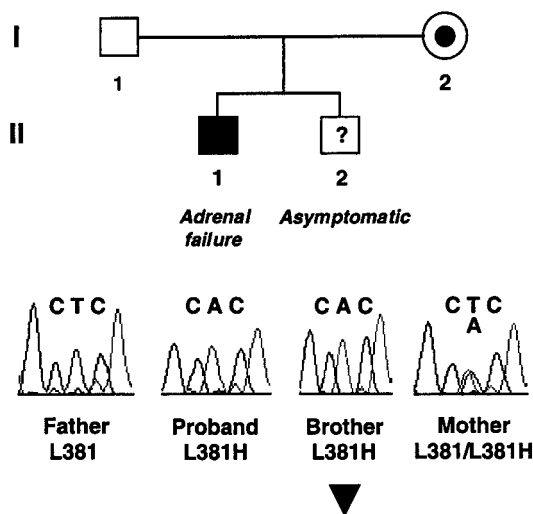
CASE REPORT: BROTHER

The 8-month-old brother had no symptoms and appeared entirely normal on physical examination, with no evidence of hyperpigmentation. Investigations of adrenal function showed normal electrolytes but evidence of compensated primary adrenal failure (Fig 1). His 11-deoxycortisol (Specific S) concentration was elevated. Basal luteinizing hormone (0.62 IU/L) and follicle-stimulating hormone (3.0 IU/L) were detectable, and testosterone (72 ng/dL [normal: <10 ng/dL]) was above the normal range for male infants of this age. These data support reports of preserved hypothalamic-pituitary gonadal function in infancy in some boys with *DAX1* gene mutations.¹⁰⁻¹²

An adrenocorticotropin stimulation test (0.25 mg intravenous corticotropin; Cortrosyn; Organon, West Orange, NJ) (Fig 1) revealed impaired adrenal reserve. However, a significant increase in 11-deoxycortisol was obtained.

DISCUSSION

The association of *DAX1* gene mutations and X-linked AHC is well established. Approximately 60% of affected boys have an early onset of primary adrenal failure, usually within the first 2 months of life. Urgent mineralocorti-



	Basal	ACTH stimulated
Cortisol (µg/dL)	16 (6-23)	18 (25-60)
Aldosterone (ng/dL)	18 (2-39)	22 (5-94)
17-OHP (ng/dL)	23 (11-106)	44 (102-267)
11-deoxycortisol (ng/dL)	860 (10-156)	1933 (135-262)
Androstenedione (ng/dL)	41 (6-28)	65 (16-77)
ACTH (pg/ml)	195 (10-60)	-
PRA (ng/dL/hr)	4068 (235-3700)	-

Fig 1. Upper panel, Identification of novel *DAX1* missense mutation (L381H) in 4-year-old proband with adrenal failure (II:1) and his younger brother; who had no symptoms (II:2). This lysine to histidine change resulted from T-to-A transversion at nucleotide position 1142 (A of ATG initiation codon being designated +1). **Lower panel**, Investigations of adrenal function in boy with no symptoms (II:2) (All assays performed by Endocrine Sciences Inc, Calabasas Hills, CA) (17-OHP, 17-hydroxyprogesterone; PRA, plasma renin activity).

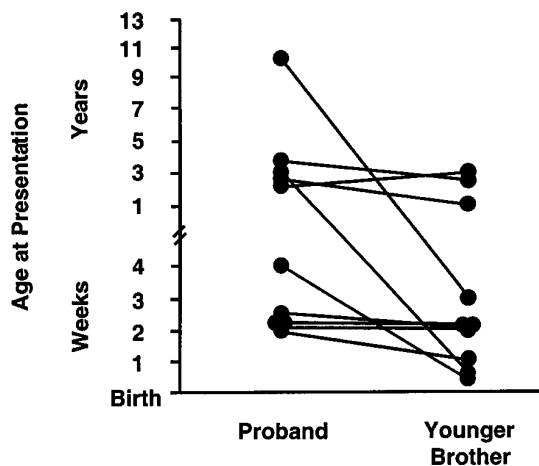


Fig 2. Age at presentation of proband and younger brother in families in which 2 siblings had adrenal insufficiency because of same *DAX1* mutation. In many cases diagnosis was reached at earlier age in younger brother ($P < .03$; Wilcoxon test). Note nonlinear scale. (Data derived from references 3, 6, 8, 12, and 14-18).

coid and glucocorticoid replacement is necessary. Other patients have a more insidious presentation later in childhood, which may be triggered by a stressful stimulus. This apparently bimodal pattern of presentation may reflect normal age-related changes in mineralocorticoid secretion and sensitivity, sodium and fluid intake, and counter-regulatory responses.⁶ Children with impaired adrenal reserve may therefore be particularly vulnerable in early life.

Families harboring *DAX1* mutations often have a history of unexplained death of maternal male relatives, highlighting the X-linked pattern of transmission. After a *DAX1* mutation is identified in a boy with adrenal failure, mutational analysis of *DAX1* and appropriate counseling are recommended for all individuals at risk of the development of AHC and for potential female carriers of child-bearing age. The case presented here exemplifies how genetic testing can be valuable even in the absence of symptoms. Identification of a *DAX1* mutation in the brother led to investigations that confirmed compensated adrenal insufficiency and impaired adrenal reserve, despite normal basal cortisol and aldosterone concentrations. Furthermore, the diagnosis of AHC predicts that affected boys will fail to enter puberty and may have impaired fertility. Consequently, appropriate counseling and hormone replacement can be instituted during adolescence.

Can the onset of adrenal failure be predicted when such children are identified? At present there appears to be little correlation between the type of *DAX1* mutation (genotype) and age at presentation (phenotype), other than the case of a man who had mild adrenal failure at 28 years of age and a *DAX1* mutation that caused partial loss of function when studied in transcription assays.¹³ Indeed, the age at presentation may vary between sibling pairs with the same *DAX1* mutation (Fig 2). Although "early presentation" or "late presentation" appears to be a feature in

certain families, the younger brother usually receives the diagnosis at an earlier age, probably reflecting increased awareness of the subtle symptoms and signs of adrenal failure by families and physicians. The use of such data to predict the age of onset of adrenal failure for siblings without symptoms who are detected by genetic screening is therefore difficult. Dynamic tests of adrenal function can be used to document adrenal dysfunction in boys with *DAX1* mutations, although experience to date suggests that all will eventually have adrenal insufficiency. Given the impaired adrenal function in this case, replacement doses of mineralocorticoid and glucocorticoid therapy were initiated, and appropriate counseling and education for steroid replacement during stress and sickness were provided.

Relatively little is known about the factors that regulate disappearance of the fetal adrenal and concomitant development of the adult-type cortex in humans. *DAX1* is clearly involved in this process, although whether it also has a direct effect on steroidogenesis remains unclear. An elevated 11-deoxycortisol has been reported in early infancy in X-linked AHC¹⁰ but never in a child this old. This finding could reflect persistent fetal adrenal function beyond the time it normally regresses or a specific impairment of 11 β -hydroxylase activity. Identification of more individuals without symptoms could provide more biochemical data on the progression of this condition. However, this report of a novel missense mutation in *DAX1* highlights the importance of testing family members at risk of *DAX1* mutations and starting steroid replacement when dynamic tests reveal impaired adrenal reserve.

REFERENCES

1. Kelch RP, Viridis R, Rapaport R, Grieg F, Levine LS, New MI. Congenital adrenal hypoplasia. *Pediatr Adolesc Endocrinol* 1984;13:156-61.
2. Zanaria E, Muscatelli F, Bardoni B,

- Strom TM, Guoli S, Guo W, et al. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature* 1994;372:635-41.
3. Muscatelli F, Strom TM, Walker AP, Zanaria E, Recan D, Meindl A, et al. Mutations in the *DAX-1* gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 1994;372:672-6.
4. Guo W, Burris TP, McCabe ER. Expression of *DAX-1*, the gene responsible for X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism, in the hypothalamic-pituitary-adrenal/gonadal axis. *Biochem Mol Med* 1995;56:8-13.
5. Yu RN, Achermann JC, Ito M, Jameson JL. The role of *DAX-1* in reproduction. *Trends Endocrinol Metab* 1998;9:169-75.
6. Reutens AT, Achermann JC, Ito M, Ito M, Gu WX, Habiby RL, et al. Clinical and functional effects of mutations in the *DAX-1* gene in patients with adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 1999;84:504-11.
7. Uttley WS. Familial congenital adrenal hypoplasia. *Arch Dis Child* 1968;43:724-30.
8. Merke DP, Tajima T, Baron J, Cutler GB. Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the *DAX1* gene. *N Engl J Med* 1999;340:1248-52.
9. Achermann JC, Gu WX, Kotlar TJ, Meeks JJ, Sabacan LP, Seminara SB, et al. Mutational analysis of *DAX1* in patients with hypogonadotropic hypogonadism or pubertal delay. *J Clin Endocrinol Metab* 1999;84:4497-500.
10. Peter M, Viemann M, Partsch CJ, Sippell WG. Congenital adrenal hypoplasia: clinical spectrum, experience with hormonal diagnosis and report on new point mutations of the *DAX-1* gene. *J Clin Endocrinol Metab* 1998;83:2666-74.
11. Takahashi T, Shoji Y, Shoji Y, Hara-guchi N, Takahashi I, Takada G. Active hypothalamic-pituitary-gonadal axis in an infant with X-linked adrenal hypoplasia congenita. *J Pediatr* 1997;130:485-8.
12. Kaiserman KB, Nakamoto JM, Geffner ME, McCabe ER. Minipuberty of infancy and adolescent pubertal function in adrenal hypoplasia congenita. *J Pediatr* 1998;133:300-2.
13. Tabarin A, Achermann JC, Recan D, Bex V, Bertagna X, Christin-Maitre S, et al. A novel mutation in *DAX1* causes

- delayed onset adrenal insufficiency and incomplete hypogonadotropic hypogonadism. *J Clin Invest* 2000;105:321-8.
14. Bassett JH, O'Halloran DJ, Williams GR, Beardwell CG, Shalet SM, Thakker RV. Novel DAX1 mutations in X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Clin Endocrinol* 1999;50:69-75.
15. Habiby RL, Boepple P, Nachtigall L, Sluss PM, Crowley WF, Jr, Jameson JL. Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalamic and pituitary defects in gonadotropin production. *J Clin Invest* 1996;98:1055-62.
16. Nakae J, Tajima T, Kusuda S, Kohda N, Okabe T, Shinohara N, et al. Truncation at the C-terminus of the DAX-1 protein impairs its biological actions in patients with X-linked adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 1996;81:3680-5.
17. Nakae J, Abe S, Tajima T, Shinohara N, Murashita M, Igarashi Y, et al. Three novel mutations and a de novo deletion mutation of the DAX-1 gene in patients with X-linked adrenal hypoplasia congenita. *J Clin Endocrinol Metab* 1997;82:3835-41.
18. Schwartz M, Blichfeldt S, Muller J. X-linked adrenal hypoplasia in a large Greenlandic family. Detection of a missense mutation (N440I) in the DAX-1 gene; implication for genetic counselling and carrier diagnosis. *Hum Genet* 1997;99:83-7.

O ***N THE MOVE?***

Send us your new address at least six weeks ahead

Don't miss a single issue of the journal! To ensure prompt service when you change your address, please photocopy and complete the form below.

Please send your change of address notification at least six weeks before your move to ensure continued service. We regret we cannot guarantee replacement of issues missed due to late notification.

JOURNAL TITLE:

Fill in the title of the journal here. _____

OLD ADDRESS:

Affix the address label from a recent issue of the journal here.

NEW ADDRESS:

Clearly print your new address here.

Name _____

Address _____

City/State/ZIP _____

COPY AND MAIL THIS FORM TO:

Mosby
Subscription Customer Service
6277 Sea Harbor Dr
Orlando, FL 32887

OR FAX TO:

407-363-9661



OR PHONE:

800-654-2452
Outside the US, call
407-345-4000