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X-Linked Adrenal Hypoplasia Congenita: A Mutation in *DAX1* Expands the Phenotypic Spectrum in Males and Females*

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ABSTRACT

X-linked adrenal hypoplasia congenita (AHC) is a disorder associated with primary adrenal insufficiency and hypogonadotropic hypogonadism (HH). The gene responsible for X-linked AHC, *DAX1,* encodes a member of the nuclear hormone receptor superfamily. We studied an extended kindred with AHC and HH in which two males (the proband and his nephew) were affected with a nucleotide deletion (501delA). The proband's mother, sister, and niece were heterozygous for this frameshift mutation. At age 27 yr, after 7 yr of low dose hCG therapy, the proband underwent a testicular biopsy revealing rare spermatogonia and Leydig cell hyperplasia. Despite steadily progressive doses of hCG and Pergonal administered over a 3-yr period, the proband remained azoospermic. The proband's mother, sister (obligate carrier), and niece all had a history of delayed puberty, with menarche occurring at ages 17–18 yr.

Baseline patterns of pulsatile gonadotropin secretion and gonadotropin responsiveness to exogenous pulsatile GnRH were examined in the affected males. LH, FSH, and free α-subunit were determined during 12.5–24 h of frequent blood sampling (every 10 min). Both patients then received pulsatile GnRH (25 ng/kg) sc every 2 h for 6–7 days. Gonadotropin responses to a single GnRH pulse iv were monitored daily to assess the pituitary responsiveness to exogenous GnRH. In the proband, FSH and LH levels demonstrated a subtle, but significant, response to GnRH over the week of pulsatile GnRH therapy. Free α-subunit levels demonstrated an erratic pattern of secretion at baseline and no significant response to pulsatile GnRH.

We conclude that 1) affected males with AHC/HH may have an intrinsic defect in spermatogenesis that is not responsive to gonadotropin therapy; 2) female carriers of *DAX1* mutations may express the phenotype of delayed puberty; and 3) although affected individuals display minimal responses to pulsatile GnRH, as observed in other AHC kindreds, subtle differences in gonadotropin patterns may nevertheless exist between affected individuals within a kindred. (*J Clin Endocrinol Metab* **84:** 4501–4509, 1999)

ADRENAL hypoplasia congenita is a rare developmental disorder of the adrenal gland. In the X-linked cytomegalic form, the adrenal glands lack the permanent adult zone of the adrenal cortex (1). Affected boys typically present with adrenal insufficiency in early infancy or childhood and hypogonadotropic hypogonadism (HH) at the time of puberty (2). The gene responsible for adrenal hypoplasia congenita (AHC) is *DAX1* (dosage-sensitive, sex reversal, adrenal hypoplasia congenita, critical region on the X-chromosome, gene 1), which encodes for a protein that is a member of the orphan nuclear hormone receptor superfamily (3, 4). Although the carboxyl-terminus of DAX-1 (putative ligand-binding domain) displays sequence homology with several other transcription factors, the amino-terminus of DAX-1 has a novel amino acid tandem repeat structure that lacks the zinc finger motif typically present in the DNA-binding domain of other nuclear hormone receptors (4). A range of frameshift and nonsense mutations has been reported throughout *DAX1* in patients with AHC and HH. These mutations cause truncation of the functionally important carboxyl-terminal region of the protein (5). Missense mutations in *DAX1* are relatively rare and, to date, are also localized within the putative ligand-binding domain alone (5). Additional lines of evidence suggest that such disruptions of the C-terminus impair DAX-1 interaction with steroidogenic factor-1 (6).

Long term data on gonadal function in patients with AHC/HH is lacking. No cases of true fertility have yet been reported in men with this condition. It is unclear whether fertility can be induced in these patients by using exogenous gonadotropins, as Dax-1 is also expressed in Sertoli cells, and the recently reported *Ahch* (*Dax1*) knockout mouse demonstrates markedly disordered spermatogenesis (7). In addition, little information has been available regarding the phenotypic status of female carriers of *DAX1* gene mutations. However, recently, a female with isolated HH was found to be homozygous for a *DAX1* nonsense mutation, demonstrating a unique molecular etiology for this disorder (8).

Much of the early clinical data on AHC patients examined whether the hypogonadism of these individuals was due to defects at the hypothalamus or pituitary. Attempts to stimulate gonadotropin secretion were conducted using pulsatile

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GnRH. Although these cases predated the discovery of *DAX1* (and, by extension, do not represent confirmed *DAX1* mutations), investigators were unable to resolve whether the hypogonadism of AHC was of hypothalamic or pituitary origin (9–13). However, by 1) conducting frequent (every 10–20 min) blood sampling for LH, FSH, and free α-subunit (FAS) to examine the baseline pulsatile activity of the hypothalamic-pituitary-gonadal (HPG) axis and 2) administering exogenous pulsatile GnRH at physiological doses to two patients with different DAX-1 mutations, we have been able to demonstrate that DAX-1 can impair gonadotropin production by acting at both hypothalamic and pituitary levels (14).

This study presents an extended kindred with AHC/HH. The proband of this family failed to develop spermatogenesis despite 3 yr of exogenous gonadotropin therapy, suggesting the presence of an independent gonadal defect in AHC/HH. In addition, this family demonstrates the novel finding that delayed puberty can be a manifestation of *DAX1* mutations in females. The results of detailed hypothalamic-pituitary-gonadal investigations in two members of this kindred are also presented.

Subjects and Methods

Case presentations

The proband, III-9 (Fig. 1), was the product of a full-term, normal, spontaneous delivery. He presented at 11 days of life with poor intake, drowsiness, and weight loss and presented again at 4 weeks with malnutrition and dehydration. At that time, examination revealed mottled skin with poor turgor, but the abdomen, genitalia, and extremities were normal. Electrolyte determinations consistently showed low sodium and chloride and elevated potassium levels. Serum cortisol levels were subnormal, with only a 2- to 3-fold increase after ACTH administration. The patient remained hospitalized for the next 2.5 months and received cortisone treatment. He was discharged with a diagnosis of transient hypoadrenalism of infancy. Although he did not require any steroid supplementation during childhood, III-9 recollects a childhood preference for salty foods. He presented at age 12 yr with anorexia, weight loss, poor growth, and hyponatremia (Na, 118 mEq/L). Physical examination was notable for hyperpigmentation, and he was begun on hydrocortisone and fludrocortisone treatment.

In his teen years, III-9 presented with delayed puberty. A presumptive diagnosis of HH was made, and hCG (1000 U, im, every 5 days) was initiated at age 19 yr. An excellent response to hCG was evident, with respect to both virilization and sexual function. He was 5 ft 4 in. when he graduated high school and 6 ft 4 in. when he graduated college. At age 27 yr, a testicular biopsy was performed, which revealed Sertoli cell only syndrome with rare spermatogonia and no apparent spermatogenesis (Fig. 2).

After the biopsy, in an attempt to achieve fertility, III-9 initiated combination therapy with hCG and Pergonal (75 U, im, three times per week). Initially, physical examination revealed a testicular size of 5 cc bilaterally. After 3 yr of therapy (dose escalations to 2000 U hCG and 150 U Pergonal, im, three times per week), testicular size increased to 10 cc bilaterally, but semen analyses failed to show any sperm. The patient discontinued gonadotropins, declined pulsatile GnRH, and initiated testosterone therapy.

The patient had five siblings, three of whom died prematurely. A 14-yr-old sister had homocystinuria (III-8), a 2.5-yr-old sister died of nephritis (III-3), and a boy born 2 months prematurely died at 2.5 days of age (III-2). The proband's brother underwent normal puberty and development. The proband's father had a history of thyrotoxicosis. His mother (II-4) had a history of delayed puberty, with menarche at age 17 yr.

The patient III-9's surviving sister (III-4), similar to her mother, also underwent a delayed menarche at age 17–18 yr. However, after completion of puberty, she maintained regular menstrual cycles and conceived two children. In 1981, she had a healthy daughter (IV-1), who had normal growth and development, but did not undergo menarche until age 17 yr. Although the history is vague, both III-4 and IV-1 underwent pubarche and thelarche in their early teens. All three women in this lineage had no history of eating disorder, marked weight loss, major life stressors, or competitive athletics, with the exception of IV-1, who ran on her high school track team.

In 1983, III-4 bore a son (IV-2) who was an 8-lb 5-oz product of an uncomplicated full-term pregnancy. He presented at 4 weeks of age with irritability, dehydration, hyponatremia, and hyperkalemia. Physical examination revealed a slightly thin but well developed male infant with normally descended testes. During hospital admission, IV-2 was treated with hydrocortisone and iv sodium chloride; fludrocortisone and deoxycorticosterone acetate were added to his regimen. Work-up revealed an elevated serum renin, low serum aldosterone, and normal serum testosterone (209 ng/dL), LH (6 mIU/mL), FSH (2 mIU/mL), and 17α-hydroxyprogesterone (352 ng/dL). Patient IV-2 was switched to dexamethasone and readmitted at 7.5 weeks for confirmation of his diagnosis with ACTH stimulation testing. A 3-day ACTH stimulation test yielded no cortisol elevation, and he was restarted on hydrocortisone and fludrocortisone, with intermittent injections of deoxycorticosterone.

Patient IV-2 remained well below the fifth percentile on his growth curve throughout infancy and childhood, with a height age equivalent to 4 yr at age 6 yr. He had two episodes of adrenal insufficiency,

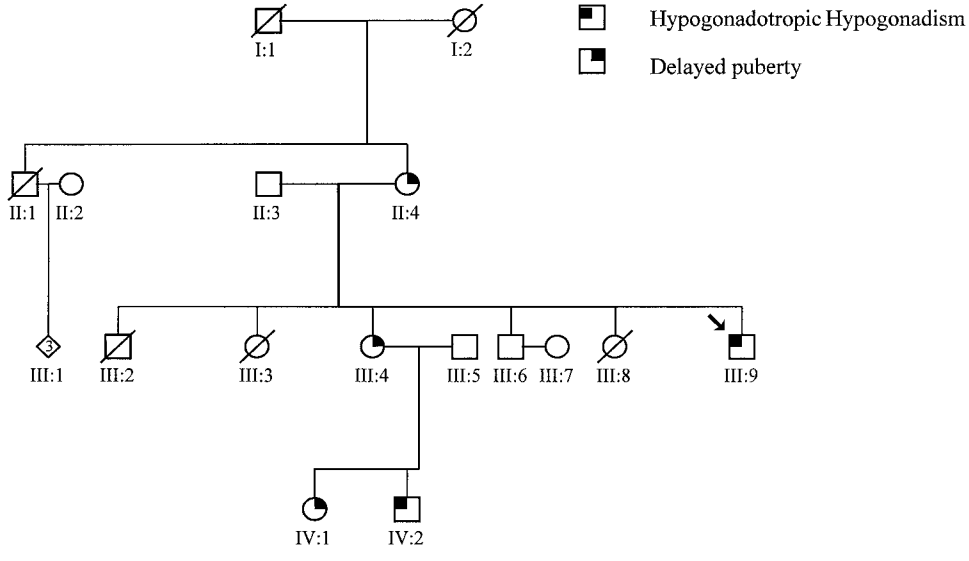

FIG. 1. Family pedigree.

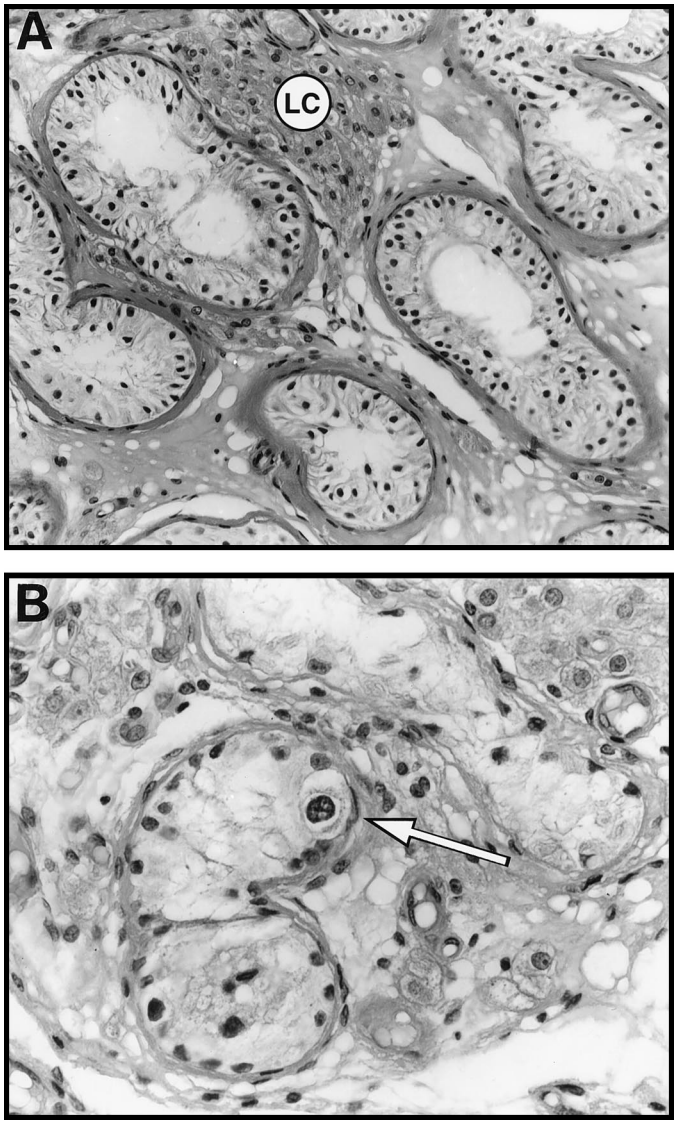

FIG. 2. Photomicrographs (A, ×200; B, × 400) of the testicular biopsy of the proband, III-9, after 5 yr of low dose hCG therapy. A, Sertoli cell only syndrome with no germ cells demonstrable within the seminiferous tubules. LC, Leydig cell hyperplasia. B, Higher magnification of single seminiferous tubule. The *arrow* demarcates one of the rare spermatogonia identified in the specimen.

presenting primarily with hypoglycemia, once at 15 months during gastroenteritis and otitis media, and once at 7 yr, with gastroenteritis, vomiting, and fever. At 9 2/12 yr, IV-2 underwent dynamic anterior pituitary testing. In response to 0.10 U/kg insulin, his GH level rose from 14 to 33 ng/mL (normal, >10). His cortisol level showed no response, with a baseline level of 11 μg/dL that failed to increase during testing. In response to 400 μg TRH, his TSH levels rose from a baseline of 1.3 μIU/mL to a peak TSH of 5.0 μIU/mL, an inadequate response (normal, increase of 10–30). His PRL rose from a baseline of 6 ng/mL to a peak level of 52 ng/mL (normal, >20 or 3 times baseline). In response to 100 μg GnRH, LH remained less than 0.5 μIU/mL and FSH less than 1.0 μIU/mL at all time points. At a chronological age of 9 2/12 yr, IV-2's bone age was 6 yr.

At 12 3/12 yr, patient IV-2 continued to be well below the fifth percentile on the normal growth curves (height, 51 in.; height age, 8 6/12 yr; predicted height using Bayley prediction tables, 68 in.). Physical examination revealed no pubic or axillary hair and prepubertal testes. Bone age remained markedly delayed at 6 6/12 to 8 yr. On repeat GnRH stimulation testing, testosterone levels at 1 and 120 min were less than 3 ng/100 mg. FSH levels rose from 2.4 μIU/mL to a peak of 4.6 μIU/mL at 120 min. LH levels rose from 0.16 μIU/mL to a peak of 1.1 μIU/mL at 60 min.

Clinical studies

After obtaining written informed consent, both patients were admitted to the General Clinical Research Center at the Massachusetts General Hospital, patient III-9 in 1980 (age 22 yr) and patient IV-2 in 1998 (age 15 yr). Patient III-9 discontinued his hCG for 8 weeks before the study, and patient IV-2 was not taking any hormone replacement therapy. Frequent blood sampling every 10–20 min was performed for 12.5–24 h for measurement of LH, FSH, and FAS. FSH levels were measured from hourly pools, and testosterone levels were measured from 6-h pools of serum. After completion of frequent sampling, each patient received pulsatile GnRH (25 ng/kg) sc via pulsatile pump every 2 h for 6–7 days. Each morning while using the GnRH pump, gonadotropin responses to a single iv GnRH bolus were monitored. LH, FSH, and FAS levels were determined every 15 min for 2 h after the GnRH pulse.

Assays

Serum LH and FSH concentrations were determined by immunoassays calibrated against the Second International Reference Preparation of human menopausal gonadotropin (hMG; WHO 71/223) (15–17). For patient III-9, the minimum detectable dose was 0.8 mIU hMG (WHO 71/223)/mL; for patient IV-2, the minimum detectable dose was 1.6 mIU hMG (WHO 71/223)/mL. Inter- and intraassay coefficients of variation were less than 10%. FAS was measured using a monoclonal antibody RIA, with a highly purified α-subunit of hCG as the standard (18, 19). Testosterone levels were also measured by RIA (20). Secretory patterns were analyzed for pulses using a modified version of the Santen and Bardin method (21). Statistical analysis was performed using a Newman-Keuls ANOVA.

Direct sequencing of DAX1

After obtaining informed consent, blood samples were taken from individuals II-4, III-4, III-9, IV-1, and IV-2, and leukocyte DNA was extracted. Exons 1 and 2 of *DAX1* were amplified by PCR using primers and conditions described previously (5). Direct sequencing of PCR products was performed using the dRhodamine terminator cycle sequencing kit (Perkin-Elmer Corp., Palo Alto, CA) and automated sequencer (model 377, PE Applied Biosystems, Foster City, CA). After detection of a mutation, additional primers were used to PCR amplify the region of interest (forward, 5′-GCTCAAAGCAAACGCACGTGGCTC-3′; reverse, 5′-GACGAAGCGCAGCGTCTTCAACAG-3′). Restriction enzyme analysis of this DNA fragment was performed using *Msp*I (Promega Corp., Madison, WI).

Results

Mutation analysis of the DAX1 gene

Direct sequencing of *DAX1* revealed a hemizygous 501delA mutation in both the proband (III-9) and his affected nephew [IV-2; Fig. 3A; A of the ATG start codon designated +1; 501delA of IV-2 previously reported (22)]. This single nucleotide deletion results in a frame shift and premature stop codon at position 263. Three women (II-4, III-4, and IV-1) from different generations of this kindred, each with markedly delayed puberty, were all heterozygous for this 501delA mutation (Fig. 3A). Deletion of this nucleotide creates a novel *Msp*I restriction site. Restriction enzyme analysis confirmed the hemizygous status of affected males and the heterozygous carrier status of females (Fig. 3B).

Baseline gonadotropin secretion

Patient III-9 (Fig. 4A) exhibited apulsatile gonadotropin secretion, with LH levels below the limits of detection of the

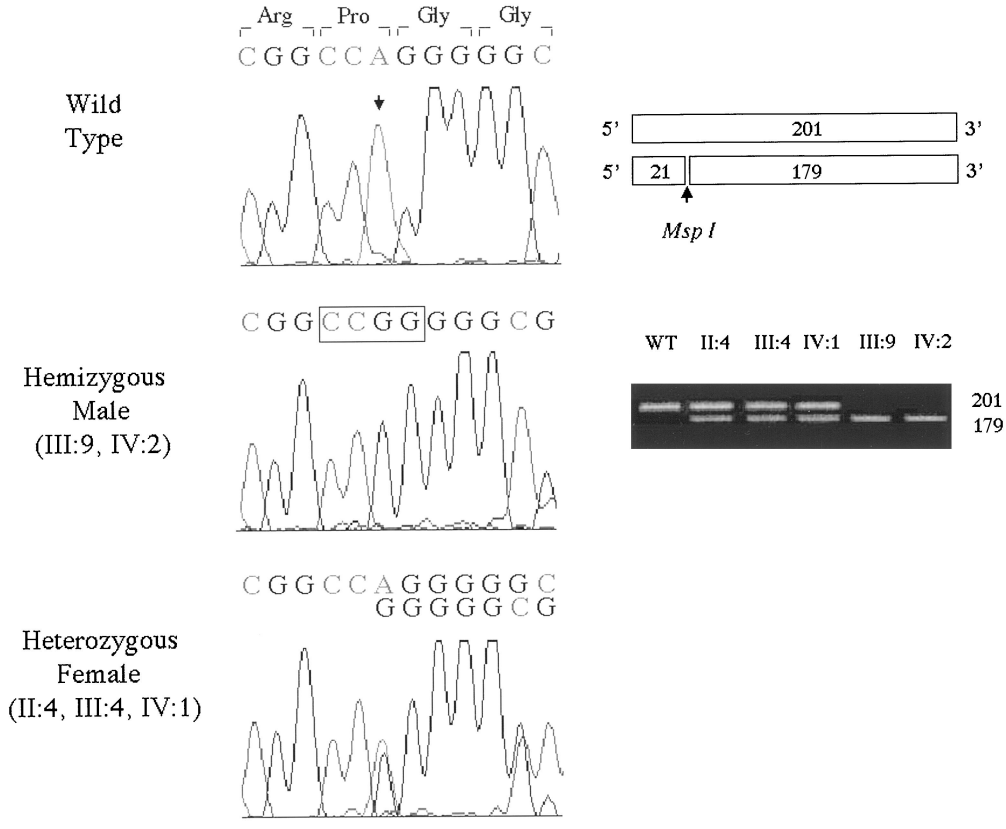

FIG. 3. Left, Direct DNA sequencing revealed a hemizygous 501delA mutation in affected males (III-9 and IV-2). Females (II-4, III-4, and IV-1) appeared heterozygous for this mutation. Right, Deletion of this nucleotide creates a novel *Msp*I restriction site (C/CGG) in the sequence. Carrier status could therefore be confirmed by restriction analysis.

assay (<0.8 mIU/mL). FSH levels were at the low end of the normal male range (mean FSH, 1.2 mIU/mL), but FAS levels were well within the normal range (mean FAS, 126.2 pg/mL). Six FAS pulses were detected by modified Santen and Bardin analysis. In patient IV-2 (Fig. 4B), LH levels were below the limits of detection (<1.6 mIU/mL) for every data point in the study. Mean FSH and FAS levels equaled 3.5 mIU/mL and 34.9 pg/mL, respectively. No FAS pulses were detectable.

Response to pulsatile GnRH

Both III-9 and IV-2 received pulsatile GnRH for 7 and 6 days, respectively, to examine their pituitary responsiveness to exogenous pulsatile GnRH (Fig. 5). There was no initial gonadotropin pulse for either patient on day 1 of therapy. For patient III-9, FSH levels tripled from day 1 to day 7 of GnRH therapy ($P < 0.001$; Fig. 5A). LH levels remained at the level of assay sensitivity for the first 4 days of the study, then increased significantly over the course of the last 3 days ($P < 0.005$). Testosterone levels increased from 6 to 141 ng/dL during the week. FAS levels did not change significantly.

Patient IV-2 demonstrated no change in LH, FAS, or testosterone levels over the course of 6 days of GnRH therapy (Fig. 5B). However, mean FSH levels increased modestly, but significantly, from day 1 to day 6 ($P < 0.001$).

Discussion

This study examines genotype/phenotype correlations in both the male and female members of an extended kindred with congenital adrenal hypoplasia. Despite extensive therapy with exogenous gonadotropins, the proband of this kindred, III-9, was not able to achieve fertility. The family history coupled with the mutation analysis strongly suggest that delayed puberty may be a manifestation of X-linked *DAX1* gene defects in female heterozygotes. Both the proband and his nephew demonstrated minimal responses to exogenous pulsatile GnRH. However, the proband demonstrated an erratic pattern of FAS secretion at baseline, as has been previously documented in patients with AHC/HH (14), whereas his nephew had no evidence of endogenous gonadotropin secretion. These observations raise a number of new issues about AHC, including 1) the variety of phenotypes included within the diagnosis, and 2) the spectrum of presentations within a family.

In the rat, Dax-1 is expressed in Sertoli cells, with maximal levels present during the first spermatogenic wave (postnatal days 20 and 30) (23). However, Dax-1 expression declines to low basal levels in the 40-day-old, sexually mature animal, demonstrating a pattern of developmental regulation. The mouse model of AHC, created by targeted disruption of the mouse homolog of *DAX1* (*Ahch*), has extended our understanding of the role of this gene mutant in gonadal function

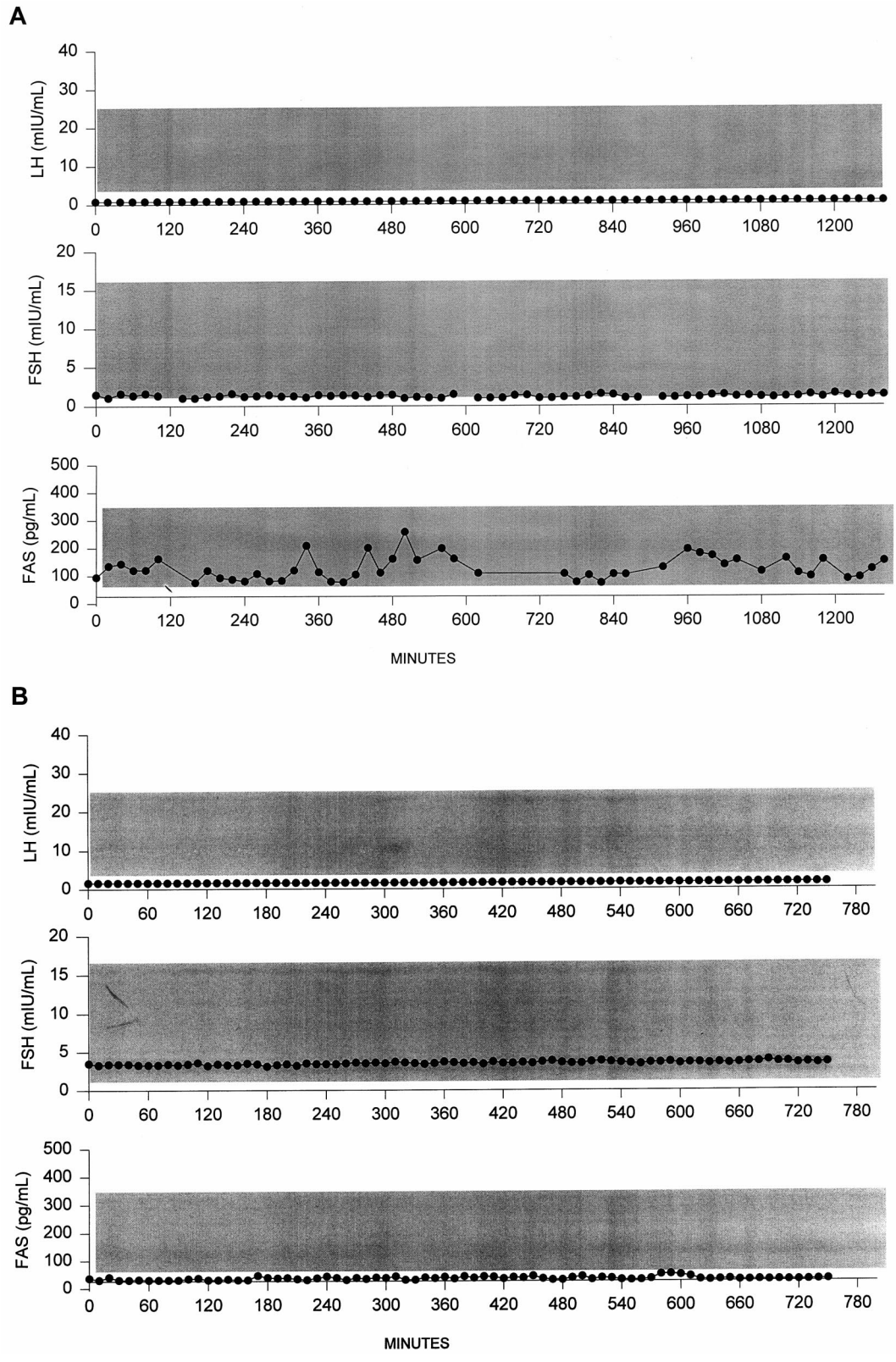

FIG. 4. Baseline secretory pattern of LH and FAS in III-9 (A) and IV-2 (B). Each graph depicts the results of gonadotropin determinations made at 10- to 20-min intervals. Patient III-9 was studied for 24 h; patient IV-2 was studied for 12.5 h. The *shaded area* for LH and FSH represents the mean ± 2 SD for each hormone as determined in 20 normal men.

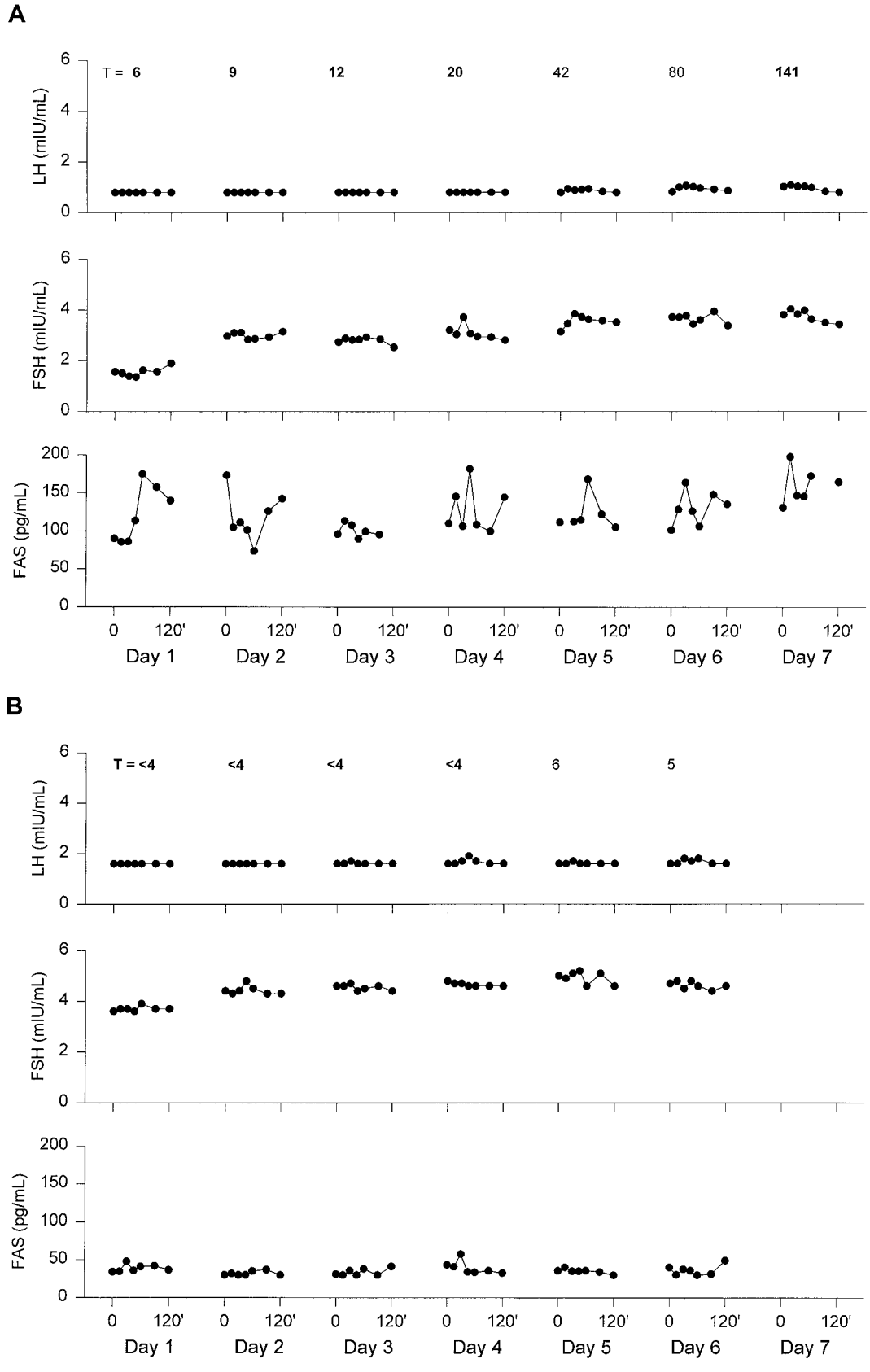

FIG. 5. Gonadotropin responses to 6–7 days of pulsatile GnRH in III-9 (A) and IV-2 (B). Each patient received pulsatile GnRH (25 ng/kg, iv, every 2 h). A single GnRH pulse was monitored each morning. Testosterone levels are indicated at the *top* of each panel. Note the change in axes compared with Fig. 4. Refer to Fig. 4 for normative ranges.

(7). Based on observations in the human, it was originally hypothesized that the hypogonadism of AHC in mice would be due to deficient gonadotropin secretion. However, mutant mice had normal levels of LH and FSH; pituitary immunohistochemical staining for LHβ and FSHβ was also normal. Male mice demonstrated a primary testicular defect with progressive seminiferous tubule epithelial degeneration, leading to a complete loss of germ cells by 14 weeks. Leydig cell hyperplasia was also observed, suggesting either an independent Leydig cell defect due to *Ahch* disruption or a secondary response to the Sertoli cell defect.

Although the differences between human and mouse phenotypes could be explained by species-specific pathways, there is a paucity of data regarding testicular histology and induction of spermatogenesis in men with AHC. Schwartz *et al.* reported the case of an affected male who underwent a short course of pulsatile GnRH and produced a semen sample with a few spermatozoa (24). However, further details regarding sperm count, morphology, and motility were not described. In the present report, the proband underwent a testicular biopsy after 7 yr of low dose hCG therapy, revealing essentially Sertoli cell only syndrome with Leydig cell hyperplasia. However, 3 yr of escalating doses of both hCG and Pergonal failed to induce spermatogenesis despite an increase in testicular size from 5 to 10 cc. These findings suggest that even when bypassing the hypothalamic-pituitary axis with exogenous gonadotropins, AHC patients can harbor an intrinsic gonadal defect. The degeneration of large segments of the seminiferous tubules observed in knockout mice suggests that Ahch is necessary for maintenance of epithelial integrity and spermatogenesis. Extending this concept to the human male, restoration of a normal hormonal milieu in AHC/HH appears unable to overcome this defect.

The age of onset of AHC appears to be variable. Most patients present with salt wasting in neonatal life, but a significant fraction are well into their childhood years before presentation (5). Although the hypogonadotropic hypogonadism associated with AHC is conventionally perceived as a congenital disorder, recent data have demonstrated transient postnatal activation of the HPG axis in AHC patients. Normal serum gonadotropin and testosterone levels have been demonstrated in a newborn male with AHC and a confirmed *DAX1* mutation (A300V) (25). Hormonal data from four boys with AHC (aged 1–3 months) have also suggested an active HPG axis (26). In the current report, the proband's nephew demonstrated a neonatal testosterone level of 209 ng/dL in the setting of a LH level of 6 mIU/mL during this same developmental window. These findings suggest that the mechanisms that control neonatal activation of the HPG axis may be distinct from those occurring at puberty or that the hypogonadotropic hypogonadism of AHC worsens over time. Therefore, our understanding of the hypogonadotropic hypogonadism associated with AHC continues to evolve, encompassing defects at the hypothalamus, pituitary, and perhaps even gonad, with congenital and developmental components.

Despite these insights into the age spectrum of AHC in male patients, until recently, little information has been available regarding the adrenal or pubertal phenotype in female carriers of *DAX1* gene mutations. Merke *et al.* reported two boys with AHC/HH and a nonsense mutation in the DNA-binding domain of the *DAX1* gene (8). The boys' maternal aunt, who was homozygous for the nonsense mutation through gene conversion, had the phenotype of isolated hypogonadotropic hypogonadism, demonstrating a surprising range of phenotypes in this one family. In the kindred reported here, three female heterozygotes carrying the del501A mutation all underwent markedly delayed menarche. Delayed puberty in female carriers may thus represent a forme fruste of the hypogonadotropic hypogonadism associated with AHC. Although it is possible that there are other etiologies for the delayed puberty in these women, no commonly implicated physiological or genetic factors could be elicited by history. Although the women in this kindred may have had an incomplete form of central hypogonadism, there is no evidence to suggest that any of them had adrenal dysfunction. The possibility that these women might have had abnormal DHEAS or aldosterone levels or diminished adrenal reserve on formal ACTH testing could not be studied.

Partial expression of X-linked disorders in heterozygous females has been well documented for a number of conditions, including ornithine transcarbamylase deficiency (27, 28), Duchenne muscular dystrophy (29, 30), and fragile X mental retardation (31). Although it is assumed that female somatic cells undergo a 50:50 random X inactivation, this is often not true in a given individual or clonal cell population. Therefore, if a female carrier has a higher proportion of mutant alleles on the active X-chromosome in a relevant cell lineage (*i.e.* skewing), she may show symptoms similar to those of a hemizygous affected male, as has been observed in hemophilia B (32). Although in the current report, multiple females within a single kindred had delayed menarche, familial nonrandom X inactivation has been described in some rare pedigrees [Duchenne muscular dystrophy (30) and hemophilia B (33)]. Although no direct evidence is presented in the current study to support familial skewing, the possibility of genetic determinants in the randomness of X inactivation remains an important model to consider (34, 35). In addition to extended pedigrees, further studies will be needed to determine whether DAX1 mutations occur in females with delayed puberty who do not have a family history of AHC/HH.

Long term data on hypothalamic-pituitary-gonadal function in patients with AHC/HH are also lacking. Age at diagnosis has been used to assess the severity of AHC (5), but no standardized biochemical testing yet appears available as a marker of reproductive phenotype. Using pulsatile GnRH therapy, investigators have observed patterns consistent with both hypothalamic and pituitary defects (9–14). In the current study, both the proband and his nephew shared many similarities with respect to their gonadotropin profiles. Neither individual demonstrated LH pulsatility at baseline. During a 1-week exposure to exogenous pulsatile GnRH, both patients demonstrated significant elevations of FSH compared to baseline levels, but the responses were very modest compared to those in patients with Kallmann syndrome, a type of HH due to an isolated hypothalamic defect in GnRH secretion. Our group has shown that Kallmann's patients given an identical regimen of pulsatile GnRH dem-

onstrate a more robust gonadotropin response, with marked increases in both LH and FSH secretion occurring within 1 week (14).

Despite these gross similarities, there were also some important differences in the gonadotropin profiles between the affected males of this kindred. Although IV-2 had flat FAS levels, III-9 had FAS levels well within the normal male range with evidence of erratic pulsatility, suggesting GnRH-independent stimulation to the gonadotrope. Patient III-9 also had significant elevations of both LH and FSH over the week of GnRH exposure, whereas IV-2 demonstrated a significant change in only FSH. Patient III-9's rise in LH, although quite subtle, was accompanied by a marked rise in his testosterone level from 6 ng/dL (day 1) to 141 ng/dL (day 7). Prior therapy with hCG may have allowed III-9 to achieve a greater mass of Leydig cells and hence an increase in testosterone compared to his nephew, although both received identical pulsatile GnRH regimens and had similarly meager LH responses. Nonetheless, one might have expected a somewhat more dramatic rise in LH to have accompanied the elevation in testosterone, raising the possibility that FSH itself may induce Leydig cell steroidogenesis or that it stimulates another factor to induce testosterone production. Although recombinant FSH does not appear to have a direct effect on testosterone production *in vitro*, recent data suggest that FSH may increase the production of a Sertoli cell-secreted factor able to up-regulate testicular steroidogenesis (36). Therefore, although both patients demonstrated atypical responses to GnRH compared to other states of GnRH deficiency, subtle differences between these two patients suggest that the proband has a less severe reproductive phenotype than his nephew.

In addition, the proband of this family survived 12 yr without glucocorticoid or mineralocorticoid replacement, suggesting that he also has a less severe form of adrenal insufficiency. Intrafamilial variability in the temporal expression and degree of adrenal insufficiency has been reported by a number of investigators (5). In a kindred previously reported by our group, the presentation of adrenal insufficiency spanned 1 day to 8 yr among affected individuals (14). The proband's nephew never had steroid therapy withdrawn after neonatal life, so it is not known whether he could have survived off of therapy. In the future, serial monitoring of ACTH levels in male patients with known DAX1-inactivating mutations may help investigators to more precisely determine the onset of adrenal insufficiency. Although most AHC patients are found to have HH when they reach pubertal age, not all patients have been found to have mutations in the *DAX1* gene, suggesting that other gene defects or epigenetic phenomena may lead to the same phenotype (3).

In summary, this study adds to the growing body of information of the hypogonadism of AHC and expands the phenotype of this disorder to include intrinsic defects in spermatogenesis in men and possibly delayed puberty in women. Both members of this kindred demonstrated gonadotropin profiles consistent with a pituitary defect, but subtle differences between the profiles suggest that the reproductive phenotype was less severe in the proband.

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