CASE REPORT

A man with a *DAX1/NROB1* mutation, normal puberty, and an intact hypothalamic–pituitary–gonadal axis but deteriorating oligospermia during long-term follow-up

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Abstract

Objective: *DAX1/NROB1* mutations cause primary adrenal insufficiency in early childhood and hypogonadotropic hypogonadism (HHG), leading to absent or incomplete sexual maturation. The aim of the study was to prospectively investigate gonadotrope and testicular functions in a patient carrying a *DAX1* mutation, who had spontaneous puberty and normal virilization but oligospermia.

Case report: The proband was referred for infertility at the age of 32 years. He reported adrenal insufficiency diagnosed at the age of 19 years. Puberty started at the age of 13 years, with spontaneous virilization, growth spurt, and testicular growth. He reported normal libido and sexual function. Physical examination showed normal virilization, penile length, and testicular volume. However, semen samples showed severe oligospermia. Hormonal measurements confirmed adrenal insufficiency but showed a preserved hypothalamic–pituitary–gonadal axis with normal testosterone and inhibin B; basal and GnRH-stimulated gonadotropin levels and LH pulsatility were also normal. He fathered a first boy by *in vitro* fertilization and a second boy without medical assistance. As a nephew also had early adrenal insufficiency, the possibility of *DAX1* mutation was raised. The same recurrent hemizygous nonsense mutation W39X was found in the proband, his nephew, and in an apparently asymptomatic brother who was found to have adrenal insufficiency, mild HHG, and azoospermia. Several evaluations of the proband over 20 years showed preserved testosterone levels and LH secretion but deteriorating oligospermia.

Conclusion: Long-term preservation of normal hypothalamic–pituitary–gonadal function in this patient, contrasting with his severe oligospermia, strongly suggests that *DAX1* is required for human spermatogenesis, independently of its known role in gonadotropin secretion.

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Introduction

DAX1 (for dosage-sensitive sex reversal, adrenal hypoplasia congenital (AHC) critical region on the X chromosome, gene 1; also called *NROB1*) mutations can cause congenital adrenal hypoplasia, a rare X-linked disorder associated with impaired development of the permanent zones of the adrenal cortex, usually revealed by primary adrenal failure in early infancy (1, 2). Hypogonadotropic hypogonadism (HHG) is also a hallmark of this disorder, usually recognized during adolescence because of the absence or interruption of normal pubertal development (1). HHG due to *DAX1* mutation results from a combination of defective hypothalamic GnRH release and defective pituitary gonadotropin production (3). Abnormal spermatogenesis is also noted in patients with

this mutation. However, it is unclear whether or not the effect of *DAX1* mutations on human spermatogenesis is due solely to the associated gonadotropin deficiency. Milder forms of the disease have been described, with adrenal insufficiency sometimes occurring in childhood or even early adulthood. A few cases of partial HHG have also been described, with clear clinical and biological signs but no subjective symptoms (4, 5). Rare cases of apparently preserved gonadotropin secretion have been reported but not fully documented in patients with *DAX1* mutations (6, 7).

Here, we report the initial features and long-term outcome of a 32-year-old man diagnosed with adrenal insufficiency at the age of 19 years, who had oligospermia but persistently normal gonadotropic function. Molecular analysis revealed the same hemizygous

Figure 1 Pedigree of the family with patients affected by AHC with mutated *DAX1* gene. Symbols represent males (squares), females (circles), affected subjects (solid symbols), carriers (dashed symbols), and the proband (arrow). DNA sequencing and mutation analysis were performed as described in (22), with minor modifications. WT, wild type. The proband is indicated by an arrow.

N-terminal nonsense *DAX1* mutation (W39X) in the proband, his young nephew and, a few years later, in his brother. The proband finally fathered two children. This is the first reported case of long-term preservation of gonadotrope function in a patient with markedly defective spermatogenesis due to *DAX1* mutation.

Case report

The proband (subject II-2, Fig. 1) was referred to the Endocrinology Department of Bicêtre Hospital, Paris, at 32 years of age for evaluation of oligospermia. Adrenal insufficiency had been diagnosed at the age of 19 years, when he presented with fatigue and repeated episodes

of sore throat and dizziness. He was treated effectively with hydrocortisone and fluorocortisone. He also reported being diagnosed with oligospermia at the age of 24 years, following failure to conceive, but denied further investigations at that time.

At the first hormonal evaluation at the age of 32 years, his height was 171 cm, his weight 83 kg, and his blood pressure 130/70 mmHg. Physical examination revealed increased skin pigmentation. Penile length was normal (10 cm). Testicular volume was 20 ml bilaterally (normal range 12–30 ml). He reported spontaneous onset of puberty at the age of 13 years, with normal virilization, growth spurt, and testicular growth. He reported normal libido, erections, and sexual function. At the time of the initial investigations, at 30 years of age, he was taking hydrocortisone 30 mg/day and 9 α -fluorocortisone 100 μ g/day. Biological and hormonal explorations confirmed the primary adrenal insufficiency (Table 1). Adrenoleukodystrophy was ruled out by a normal level of C24/C22 very-long-chain fatty acids. Computed tomography showed bilateral adrenal atrophy. During this period, the proband's sister (subject II-1, Fig. 1) gave birth to a boy who had normal descended testes and normal genitalia (subject III-1, Fig. 1) but who had an adrenal crisis (Na 122 mmol/l, K + 7.7 mmol/l) during the second week of life. The newborn had elevated plasma ACTH and renin levels, confirming primary adrenal insufficiency. The possibility of a *DAX1* mutation was therefore raised and molecular studies of the patient and his nephew were performed with the patient's and parents' informed consent. A nonsense loss-of-function (8) mutation in the *DAX1* gene, resulting in an amino-terminal truncated protein (W39X: TAG $\rightarrow$ TGG), was found in both subjects. The same mutation was found

Table 1 Hormonal and gonadal evaluation of the proband (subject II-2 in Fig. 1) at diagnosis and during follow-up. The brother (subject II-3 in Fig. 1) was evaluated at diagnosis.

	Proband							Brother	Normal range
	1987 (23 years)	1997 (32 years)	2000 (35 years)	2002 (37 years)	2003 (39 years)	2008 (43 years)	2012 (47 years)	2011 (36 years)	
Cortisol (0800 h)	–	0.5	–	0.6	0.6	<0.2	0.5	10	9–23 (μ g/100 ml)
ACTH (0800 h)	–	2315	–	1671	1414	2843	2014	749	10–50 (pg/ml)
DHEAS	–	361	–	249	268	200	–	147	800–4500 (ng/ml)
Renin (supine/ upright)	–	464/690	–	–/320	310/310	491/–	500	–	3–16/3–33 (pg/ml)
Aldosterone (supine/upright)	–	75/76	–	–/65	60/70	13/–	30	12/28	15–150/35–350 (pg/ml)
Testosterone	7.7	5.5	6.1	5.2	5.4	4.2	5.3	2.4	3.5–8.5 (ng/ml)
LH (basal/ stimulated ^a)	6.3/–	2.4/9.4	1.7/8.6	–	1.3/10.5	2.6/7.5	1.8/–	2/5.6	1.5–12.4/8.0–25 (IU/l)
FSH (basal/ stimulated ^a)	5.8/–	4.1/5.6	3.4/5.0	–	1.2/4.0	4.4/8.7	4.4/–	5.9/7.3	1.7–8.6/2.0–9.0 (IU/l)
Inhibin B	–	–	148	123	113	38	–	74	80–270 (pg/ml)
Sperm count	4 $\times$ 10 ⁶	2.3 $\times$ 10 ⁶	0.7 $\times$ 10 ⁶	0.05 $\times$ 10 ⁶	–	–	–	0	>20 $\times$ 10 ⁶ /ml

Serum LH and FSH were measured by immunofluorometric assay, testosterone levels by RIA, and inhibin B levels with an ELISA method as described previously (24). –, not done.
^aGNRH (100 μ g i.v.), the GNRH challenge test was performed as reported in (24). Plasma renin, aldosterone, DHEAS, ACTH, and cortisol levels were determined with commercial RIA kits.

Figure 2 (A) Endogenous serum LH levels (closed symbols) in the proband (subject II-2, Fig. 1) at an 11-year interval. LH pulsatility was evaluated at 32 years (upper panel) and 43 years (middle panel). LH was measured at 10-min intervals for 4 h and analyzed with the Thomas algorithm (24). Asterisks denote detectable LH pulses. LH, total testosterone, and inhibin B were measured as indicated in Table 1. (B) Endogenous serum LH levels in the proband's brother (subject II-3, Fig. 1). LH pulsatility was evaluated at 36 years. LH was measured at 10-min intervals for 4 h and analyzed with the Thomas algorithm (24). No LH pulses were detected. LH, total testosterone, and inhibin B were measured as indicated in Table 1 and in (24). TV, testicular volume.

in the unaffected proband's mother (subject I-1, Fig. 1) and sister (subject II-1, Fig. 1), and both were heterozygous.

Results of longitudinal evaluation of the proband are shown in Table 1 and Fig. 2A. The plasma total testosterone level remained normal throughout the 25 years of follow-up, indicating LH-driven preserved Leydig cell function. By contrast, oligospermia tended to worsen over time (Table 1). Likewise, inhibin B levels showed a fall over time, indicating impaired Sertoli cell function. We also observed a decrease in testicular volume (15 ml in 2012).

Analysis of endogenous pulsatile LH secretion at the age of 32 years showed a normal mean LH level and a preserved frequency (two pulses/4 h, normal $4 \pm 0.8/8$ h). Eleven years later, at the age of 43 years, testosterone and LH secretion showed a similar pattern, further indicating preserved gonadotropic function (Fig. 2A).

Because the testosterone and gonadotropin levels were normal, hormonal treatment was not proposed. After genetic counseling, the couple was offered reproductive assistance because of severe oligospermia. The patient fathered a first healthy son (subject III-3, Fig. 1) at the age of 33 years, after *in vitro* fertilization of his wife's oocytes. A second healthy son was born 2 years later (subject III-4, Fig. 1), after spontaneous conception. The younger brother (subject II-3, Fig. 1) denied evaluation until the age of 36, when he was referred to the Endocrinology Department of Ambroise Paré Hospital for exploration of azoospermia. Physical examination revealed normal weight and blood pressure, complete virilization, and normal penile length (11 cm) and testicular volume (25 ml). He reported normal sexual function. The testosterone level was rather low with a subnormal LH response to GnRH stimulation (Table 1) and apulsatile LH secretion (Fig. 2B). Blood electrolytes were normal. However, an abnormal cortisol response to the standard-dose (250 µg i.v.) cortrosyn test (peak: 14.0 µg/100 ml, $n > 27$), elevated ACTH, and a low supine aldosterone plasma level revealed mild asymptomatic adrenal insufficiency. Hydrocortisone and fludrocortisone administration were recommended. DAX1 analysis confirmed that he carried the W39X familial mutation. After 5 months of combined gonadotropin treatment, his total testosterone was normal, 7.3 ng/ml (N; 2.5–8.4), his FSH serum level was 2.3 IU/l, and his inhibin B level normalized to 139 pg/ml but azoospermia persisted.

Discussion

We describe a man with a DAX1 nonsense mutation associated with late-onset adrenal insufficiency and normal gonadotropic function but severe oligospermia. DAX1-mutated patients usually have severe adrenal

insufficiency diagnosed during the first weeks of life (9) or in childhood. In rare cases, onset occurs in early adulthood (4, 10, 11), sometimes with prominent effects on mineralocorticoid function (6, 8, 9, 10). The phenotypes of the proband, his brother, and his nephew illustrate the different possible adrenal consequences of identical *DAX1* mutations (12, 13). The adrenal phenotypes of the proband and his brother are reminiscent of the mouse model, in which adrenal failure is a late event: after a transient period of enhanced adrenal susceptibility to ACTH, aging *Dax1* knockout mice display decreased adrenal steroidogenic capacity and adrenal cell proliferation (14). We do not know whether the proband and his brother had a period of elevated adrenal steroid hormone production before adrenal failure. However, long-term follow-up of subject II-3, who has partly preserved adrenal function at the age of 35 years, will show whether adrenal steroidogenic function declines further with aging. This would argue for a role of *DAX1* in the maintenance of adrenal progenitor cells in humans, as in mice.

The gonadotropic phenotype of this patient is most unusual. HHG in boys with *DAX1* mutations is usually severe and revealed by absent or impaired pubertal development. Some patients also have unilateral or bilateral cryptorchidism (9), indicating that gonadotropin secretion has been deficient since as early as intrauterine life (15). The most striking feature of the proband reported here is the absence of hypogonadism, with normal puberty and persistently normal spontaneous LH pulsatile secretion and testosterone levels. Some reports have described partial pubertal development and oligospermia in rare patients with *DAX1* mutations, in whom low serum testosterone concentrations indicated partial gonadotropic deficiency (4, 10). More recently, men with missense mutations located in the amino-terminal portion of *DAX1* (W105C and C200W) were reported to have an apparently normal hypothalamic–pituitary–gonadal axis with no signs or symptoms of hypogonadism (6, 7). In these families, the disease was revealed by mild adrenal disorders in the affected probands, whereas male relatives carrying the same mutation seemed to have apparently normal pubertal development and sexual function (6, 7). However, the pathophysiological effect of the C200W mutation is still unclear, and overall, these reports did not include exhaustive clinical data on the reproductive phenotype, or detailed investigations of pituitary gonadotropic and testicular hormone secretion. In addition, no specific information was provided on testicular exocrine function (sperm count) in these ‘asymptomatic’ men, or on the natural history of their reproductive function (6, 7).

As far as we are aware, this is the first report of a *DAX1* mutation in a patient with documented normal gonadotrope function (normal testosterone secretion, normal basal and stimulated LH secretion and pulsatility, and normal testicular volume). By contrast,

this patient exhibited impaired spermatogenesis, with gradually worsening oligospermia and Sertoli cell endocrine dysfunction. This association between a normal gonadotrope axis and severe testicular dysfunction further suggests that, as in mice, *DAX1* deficiency could cause progressive alteration of the testicular germinal epithelium, independently of gonadotropin and testosterone production. Studies of *Ahch* (*Dax1*) knockout mice (16) and patients with AHC (3) had already suggested that *DAX1* mutations could directly affect spermatogenesis. First, targeted disruption of *Ahch* (*Dax1*) in mice results in infertility despite apparently normal gonadotropin and adrenal steroid production (16). Evaluation of the male reproductive tract in *Dax1*-deficient mice by light and electron microscopy revealed that the rete testis is blocked by aberrantly located Sertoli cells, creating a tailback of necrosing sperm (16).

Secondly, azoospermia has been reported in most patients with classical X-linked AHC caused by *DAX1* mutations, and attempts to induce fertility with exogenous gonadotropins or GnRH have been unsuccessful (8, 17, 18). Thus, this near-universal failure of gonadotropin treatment to induce spermatogenesis in men with classical *DAX1* phenotype associated with the *Dax1* mice phenotype are in favor of the hypothesis of a direct deleterious effect of *DAX1* mutations on human spermatogenesis. Our case therefore further indicates that the mouse model is relevant to human pathology.

As indicated earlier, primary testicular dysfunction in patients with *DAX1* mutation is usually considered to be a defect that cannot be restored by gonadotropin treatment. However, a case of paternity after TESE–ICSI was recently described in a patient with a *DAX1* mutation and classical AHC, HHG, and azoospermia (19), giving hope to men with such mutations. Interestingly, the proband described here fathered a child at 35 years of age, when his sperm count was only 0.7 million/ml. It has previously been demonstrated that conception can occur despite a very low sperm count, as for example in patients with HHG receiving gonadotropin therapy (20). The defective spermatogenesis in this patient is worsening with age, suggesting that semen preservation should be offered to young men with *DAX1* mutations and mild spermatogenesis.

Most *DAX1* mutations described so far (6, 21, 22) lead to an absent or truncated protein, resulting in a severe phenotype. Missense mutations, reported in about 20% of cases, are associated with more variable reproductive phenotypes (4, 6, 10, 12). The recurrent (5, 11) W39X mutation found in our patient and in his affected brother leads to an amino-truncated *DAX1* protein generated from an alternative in-frame translation start site and retaining partial activity (8). As previously reported for another amino-terminal nonsense close mutation, Q37X, the W39X mutation was also associated with a mild phenotype (5, 8). Thus, it has been reported that gonadotrope function is often preserved in patients with mutations resulting in a

N-terminal truncated DAX1 protein (5, 6, 7, 8) that may provide sufficient DAX1 activity for gonadotrope cell development and function. We must, however, specify that the W39X mutation can also be associated with partial or complete gonadotropin deficiency as in the proband's brother described here or in the patients reported by Guclu *et al.* (11).

Testicular biopsy has been performed in a small number of DAX1-mutated men, showing diverse histological aspects (8, 17, 19, 23). The first biopsy reported in an adult male was performed at the age of 27, following 7 years of hCG treatment. It revealed a Sertoli cell-only syndrome aspect with rare spermatogonia and no apparent ongoing spermatogenesis (17). A second report concerned a 20-year-old patient with a milder phenotype who had been treated for 6 months with exogenous gonadotropins (8). Biopsy revealed disorganization of the normal tubule structure and abnormal proliferation of interstitial tissue, as well as moderate Leydig cell hyperplasia, possibly related to chronic hCG administration. Interestingly, postmortem examination of a neonate who died at 23 days revealed normal testicular histology (23) with the presence of well-defined seminiferous testis cords containing numerous Sertoli cells and germ cells. This is compatible with a progressive deterioration of testicular function due to DAX1 mutations, as observed in our patient. Finally, Frapsauce *et al.* recently reported severe hypospermatogenesis in an adult, with a majority of germ cells arrested at the spermatocyte stage. Although most of the tubules were depleted of mature spermatozoa, some tubules exhibited focal complete spermatogenesis and rare mature spermatozoa that could be retrieved from mechanically dilacerated testicular tissue and used to successfully perform TESE/ICSI (19).

In conclusion, this case report further extends the clinical phenotype of DAX1 mutations to include isolated infertility with normal pubertal development and long-term integrity of the hypothalamic–pituitary–gonadal axis. The association of this phenotype with an adrenal insufficiency should evoke a DAX1 mutation. The severely impaired spermatogenesis in this patient adds direct evidence to the argument that in humans, as in rodents, DAX1 affects testicular function independently of its effects on gonadotropin secretion. This and other observation indicates that DAX1 mutations do not always result in male sterility but we show here that semen preservation should be offered to young men with DAX1 mutations and mild spermatogenesis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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