

# Birth after TESE–ICSI in a man with hypogonadotropic hypogonadism and congenital adrenal hypoplasia linked to a DAX-1 (*NR0B1*) mutation

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Submitted on July 4, 2010; resubmitted on November 30, 2010; accepted on December 3, 2010

**ABSTRACT:** *DAX1/NR0B1* mutations are responsible for X-linked congenital adrenal hypoplasia (AHC) associated with hypogonadotropic hypogonadism (HH). Few data are available concerning testicular function and fertility in men with *DAX1* mutations. Azoospermia as well as failure of gonadotrophin treatment have been reported. We induced spermatogenesis in a patient who has a *DAX1* mutation (c.1210C>T), leading to a stop codon in position 404 (p.Gln404X). His endocrine testing revealed a low testosterone level at 1.2 nmol/l (N: 12–40) with low FSH and LH levels at 2.1 IU/l (N: 1–5 IU/l) and 0.1 IU/l (N: 1–4 IU/l), respectively. Baseline semen analysis revealed azoospermia. Menotropin (Menopur®:150 IU, three times weekly) and human chorionic gonadotrophin (1500 IU, twice weekly) were used. After 20 months of treatment, as azoospermia persisted, bilateral multiple site testicular biopsies were performed. Histology revealed severe hypospermatogenesis. Rare spermatozoa were extracted from the right posterior fragment and ICSI was performed. Four embryos were obtained and, after a frozen–thawed single-embryo transfer, the patient's wife became pregnant and gave birth to a healthy boy. We report the first case of paternity after TESE–ICSI in a patient with *DAX1* mutation, giving potential hope to these patients to father non-affected children. Furthermore, this case illustrates the fact that patients with X-linked AHC have a primary testicular defect in addition to HH.

**Key words:** hypogonadotropic hypogonadism / *DAX1* / assisted reproductive techniques / congenital adrenal hypoplasia (AHC) / genetic counselling

## Introduction

*DAX-1* [Dosage sensitive sex-reversal, Adrenal hypoplasia congenita, on the X chromosome gene 1], alternatively called *NR0B1*, is a gene that encodes an orphan member of the nuclear hormone receptor family (Zanaria *et al.*, 1994). The protein translated from this gene, called DAX-1, is essentially expressed in tissues involved in steroid hormone production and reproductive function such as the adrenals, hypothalamus and pituitary, as well as the testes (Guo *et al.*, 1995). Clinically, *NR0B1/DAX1* mutations are responsible

for X-linked congenital adrenal hypoplasia (AHC) (MIM # 300200) associated with hypogonadotropic hypogonadism (HH) (Lin *et al.*, 2006). In mice, *Nr0b1* null homozygous male mice have adrenal insufficiency as well as testicular disorganization, dilated seminiferous tubules and failed spermatogenesis (Park *et al.*, 2005). The dilated tubules in the adult reflect impaired testis cord morphogenesis during embryonic development (Meeks *et al.*, 2003). Histological examination revealed a progressive degeneration of seminiferous tubule epithelium, hyperplasia of Leydig cells and sloughing of germ cells. A Sertoli-cell-specific expression of a *DAX1* transgene is sufficient

to partially rescue the primary testicular defect of the male *Dax-1*-deficient mouse (Jefferis *et al.*, 2001).

Relatively few data are currently available concerning the testicular function and fertility of men with X-linked AHC harbouring *DAX1* mutations. Some cases of azoospermia as well as failure of gonadotrophin treatment have been described (Mantovani *et al.*, 2002; Brown *et al.*, 2003; Ozisik *et al.*, 2003; Seminara *et al.*, 1999). Oligospermia has been reported in a rare case of late-onset AHC associated with a partial loss-of-function mutation (Tabarin *et al.*, 2000). The failure of the gonadotrophin treatment described was judged on semen quality, and open testicular biopsy had not been attempted previously in order to treat male infertility.

We report the first birth of a child after a successful assisted reproductive technique (ART) in a man with classic AHC and HH due to a *DAX1* mutation, using TESE–ICSI.

## Case report

Our patient presented with primary adrenal insufficiency at the age of 3 weeks, diagnosed on account of major dehydration. He was treated with hydrocortisone and 9- $\alpha$ -fludrocortisone in order to restore glucocorticoid and mineralocorticoid deficiencies. He was referred to our unit at the age of 19 years because of hypogonadism. He had no history of cryptorchidism and his testes were small, 6 ml on each side. His penis was 6 cm in length and he had sparse pubic hair. His sense of smell was normal and he was shaving twice a month. His puberty was evaluated at P2 Tanner's stage. Bone densitometry revealed osteopenia with a T-score of  $-2.5$  DS at the lumbar level. His bone age reached 15 years. His testosterone level was low 1.2 nmol/l (N: 12–40). Baseline FSH and LH levels were 2.1 IU/l (N: 1–5 IU/l) and 0.1 IU/l (N: 1–4 IU/l), respectively. A GnRH test (100  $\mu$ g, subcutaneous) was conducted. The FSH and LH peaks reached 3.1 and 0.9 UI/l, respectively. LH and FSH pulsatility was studied over a 4-h period. No LH nor FSH pulses could be detected. Prolactin and FT4 levels were normal at 14.6 pg/ml (N: 5–10) and 17.2 pmol/l (N: 10–23), respectively. Pituitary MRI was normal. An adrenal scan revealed an atrophic left adrenal. The right adrenal could not be visualized. In summary, he presented with adrenal insufficiency associated with HH. He is an only child and a sporadic case in his family.

Considering the association of adrenal insufficiency and HH, genetic testing of the *NROB1/DAX1* gene was conducted, after obtaining informed consent according to the French ethics law. As shown in Fig. 1, a change in nucleotide sequence (c.1210C>T) was identified, leading to a stop codon in position 404 (p.Gln404X). This mutation in our patient is found in a large cohort of AHC patients (Lin *et al.*, 2006).

Hypogonadism was treated by testosterone enanthate (Androtardyl<sup>®</sup>: 250 mg intramuscularly, every 3 weeks). Six years later, as he desired paternity, a semen analysis was performed, which revealed azoospermia. Semen volume and semen pH were found to be normal. Spermatogenesis was therefore induced by gonadotrophins using menotropin (Menopur<sup>®</sup>: 150 IU of FSH and 150 IU of LH, three times weekly) and human chorionic gonadotrophin (hCG: 1500 IU, twice weekly). One month later, his testosterone level reached 4 ng/ml. The treatment doses remained constant. After 20 months of treatment, testis volume reached 11 and 12 ml, for his

right and left testis, respectively. However, azoospermia persisted. Consequently, bilateral testicular biopsies were performed. It was a blind, multiple-site biopsy. Very few spermatozoa were extracted from the right posterior fragment. Three straws were cryopreserved, each containing 100 motile spermatozoa. Histological examination revealed severe hypospermatogenesis. The diameter of the seminiferous tubules was either normal or reduced. Tubules were limited by a thickened basement membrane and contained mostly Sertoli cells and a few germ cells arrested at the spermatocyte stage (Fig. 2).

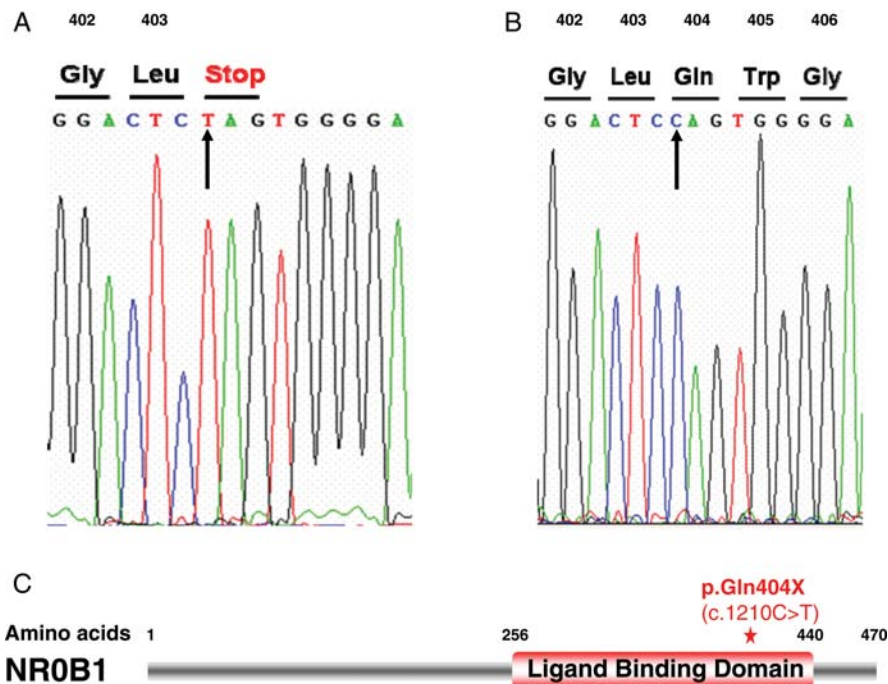
His wife had been stimulated using triptorelin (Decapeptyl<sup>®</sup>) and recombinant FSH (Gonal-F<sup>®</sup> 150 IU) for 10 days. As a result, 11 oocytes were obtained, seven of them being mature. ICSI was performed using all the cryoconserved straws. No motile spermatozoa were recovered after thawing, and thus pentoxifylline (1 mg/ml) was used. Indeed, after the freezing–thawing process, the percentage of motile testicular spermatozoa is usually low, with a weak motility making the selection of live spermatozoa difficult. Pentoxifylline was found to significantly increase the motility of spontaneously immotile epididymal and testicular spermatozoa and allows fertilization, pregnancy and birth after ICSI (Terriou *et al.*, 2000). Selection of viable spermatozoa is therefore easier. Four embryos were obtained: two Day-2 embryos were transferred and the remaining two were cryopreserved. No pregnancy followed. The following cycle, only one embryo remained viable after thawing and was used in a single-embryo transfer during a stimulated cycle. After this procedure, the patient's wife became pregnant and gave birth—9 months later, by Caesarean section—to a healthy boy.

## Discussion

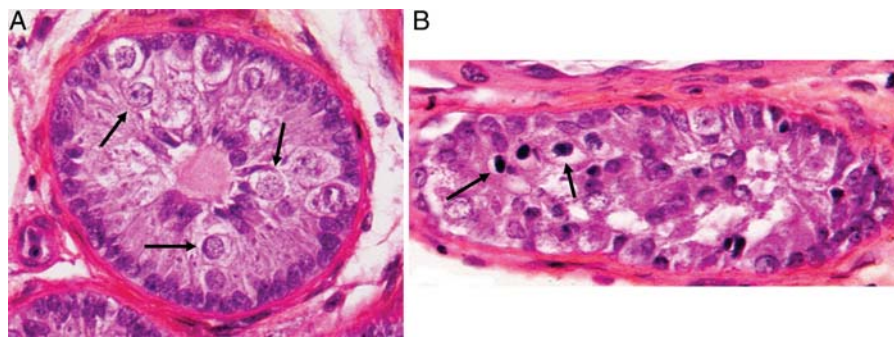
We report the first case of a child born after TESE–ICSI, with the sperm induced by gonadotrophin treatment, in a man with classic X-linked AHC due to a nonsense mutation in *NROB1/DAX1*.

Our patient had a severe clinical phenotype of neonatal adrenal insufficiency associated with hypogonadism, rather than a delayed onset or milder form of AHC (Brown *et al.*, 2003; Ozisik *et al.*, 2003). In this case, the c.1210C>T nucleotide change is predicted to result in a p.Q404X nonsense mutation located in the C-terminal ligand-like binding domain of the protein (Lin *et al.*, 2006). Missense mutations within this domain have already been shown to induce lower activity of *DAX1* (Tabarin *et al.*, 2000) and the p.Q404X change is likely to escape nonsense-mediated decay and to result in a truncated protein product lacking an AF-2 domain (Fig. 1C). Therefore, the nonsense mutation in our patient was considered to be responsible for his severe phenotype.

Gonadal defects in men carrying *DAX1* mutations have been described in several cases (Caron *et al.*, 1999; Tabarin *et al.*, 2000; Achermann *et al.*, 2001; Mantovani *et al.*, 2002). In our patient, a few testicular spermatozoa were obtained from a testicular biopsy after a long period of treatment using exogenous gonadotrophins. In the past 20 years, such treatments have been used to induce spermatogenesis in men with isolated HH (Finkel *et al.*, 1985; Büchter *et al.*, 1998; European Metrodin HP Study group, 1998; Matsumoto *et al.*, 2008; Liu *et al.*, 2009). Sperm are usually obtained after 12–18 months of treatment. Gonadotrophin treatment has been used to induce or improve fertility in men with X-linked AHC with limited



**Figure 1** (A) Nonsense p.Q404X (Gln404Stop) mutation in *DAX1*. (B) Control sequence (arrow: nucleotide exchange). (C) The mutation, as shown by a red star, is located in the ligand-like binding domain leading to a premature stop codon and predicted non-functional protein.



**Figure 2** Seminiferous tubule sections showing (A) Sertoli cells only or an arrest of spermatogenesis at the spermatocyte stage (arrow) in most tubules, and (B) isolated tubules with very rare spermatids (arrow). Few normal Leydig cells were seen. Magnification  $\times 400$ .

success (Seminara et al., 1999; Mantovani et al., 2002; Brown et al., 2003; Ozisik et al., 2003; Mantovani et al., 2006). A patient with a mild phenotype, described by Tabarin et al. (2000), had oligospermia that did not respond to gonadotrophin treatment. In all the other men treated, persistent azoospermia was observed after 6, 8, 9, 12 and even 36 months of treatment with exogenous gonadotrophins (Seminara et al., 1999; Mantovani et al., 2002; Brown et al., 2003; Ozisik et al., 2003; Mantovani et al., 2006). In our patient, gonadotrophins were administered for 20 months and azoospermia was found to persist upon semen analysis. His FSH, LH and testosterone serum levels were regularly checked and were normal, implying compliance with gonadotrophin injections. This suggests an intrinsic role for

*DAX1* in the adult human testis resulting in a defect that cannot easily be restored by gonadotrophin treatment. However, the treatment may well have allowed the development of a small number of spermatozoa that were obtained following testicular biopsy and used for ICSI.

In mice, targeted deletion of *Dax1* (*Ahch*) revealed abnormalities in testis development, in rete testis and in spermatogenesis (Park et al., 2005). Efferent duct epithelium was hyperplastic and seminiferous tubules were obstructed by ectopic Sertoli and Leydig cells in adult mice (Jefferis et al., 2001). Therefore, *DAX-1* is not needed for initiating spermatogenesis but seems necessary to induce regular spermatogenesis.

In humans, few testicular biopsies have been reported so far (Seminara *et al.*, 1999; Brown *et al.*, 2003; Ozisik *et al.*, 2003). The first biopsy reported in an adult male was performed at the age of 27, following 7 years of low-dose hCG treatment. It revealed Sertoli-cell-only syndrome, rare spermatogonia and no apparent spermatogenesis (Seminara *et al.*, 1999). A second report was of a 20-year-old patient after 6 months of treatment with exogenous gonadotrophins. This patient carried an N-terminal *DAX-1* nonsense mutation (p.Q37X) and had a milder phenotype than our patient as he presented with adrenal insufficiency at the age of 20 (Ozisik *et al.*, 2003). The testicular biopsy revealed disorganization of the normal tubule structure and abnormal proliferation of the interstitial tissue as well as moderate Leydig cell hyperplasia. Interestingly, post-mortem testicular histology from a neonate who died at 23 days has revealed normal testicular histology (Brown *et al.*, 2003) with the presence of well-defined seminiferous testis cords containing numerous Sertoli cells and germ cells. This suggests a potential progressive defect in testicular function over the years.

In our patient, histology shows severe hypospermatogenesis with most of the germ cells arrested at the spermatocyte stage. Hypospermatogenesis is characterized by a low, basal level of spermatozoid production, with most of the tubules being depleted of mature forms of spermatozoa (Fig. 2). However, some tubules may contain focal complete spermatogenesis that is not visible in the thin paraffin section stained by hematoxylin and eosin. However, these rare mature spermatozoa may be retrieved from mechanically dilacerated testicular tissue. The testicular phenotype of our patient confirms data obtained in mice, suggesting that patients with *DAX1* mutations have a primary testicular defect in addition to central HH. Our case report suggests that a testicular biopsy should be considered if patients remain azoospermic after gonadotrophin treatment.

Genetic counselling in couples where the man is affected by an X-linked condition should mention that every male offspring will be unaffected and that every female offspring will be a heterozygous carrier of the mutation. Consequently, the risk of having an affected male is moved to the second generation. Couples should be aware of sex selection of embryos by preimplantation genetic diagnosis if they wish to avoid this risk.

In conclusion, our case report gives potential hope to patients with *DAX1* mutations to father non-affected children after TESE–ICSI.

## Acknowledgements

We would thank Docteur Michèle Guittard for referring the patient to our unit and Prof. Jean-Marie Antoine for performing testicular biopsies

## Funding

J.C.A. is a Wellcome Trust Senior Fellow in Clinical Science (079666). Funding to pay the Open Access publication charges for this article was provided by Wellcome Trust, UK.

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