CASE REPORT

Pubertal androgenization and gonadal histology in two 46,XY adolescents with NR5A1 mutations and predominantly female phenotype at birth

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Abstract

Objective: Most patients with NR5A1 (SF-1) mutations and poor virilization at birth are sex-assigned female and receive early gonadectomy. Although studies in pituitary-specific Sf-1 knockout mice suggest hypogonadotropic hypogonadism, little is known about endocrine function at puberty and on germ cell tumor risk in patients with SF-1 mutations. This study reports on the natural course during puberty and on gonadal histology in two adolescents with SF-1 mutations and predominantly female phenotype at birth.

Design and methods: Clinical and hormonal data and histopathological studies are reported in one male and one female adolescent with, respectively, a nonsense mutation (c.9T>A, p.Tyr3X) and a deletion of the first two coding exons (NCBI36/hg18 Chr9:g.(126306276-126307705)_(126303229-126302828)del) of NR5A1, both predicted to fully disrupt gene function.

Results: LH and testosterone concentrations were in the normal male range, virilization was disproportionate to the neonatal phenotype. In the girl, gonadectomy at 13 years revealed incomplete spermatogenesis and bilateral precursor lesions of testicular carcinoma in situ. In the boy, at the age of 12, numerous germ cells without signs of malignancy were present in bilateral testicular biopsy specimen.

Conclusions: In SF-1 mutations, the neonatal phenotype poorly predicts virilization at puberty. Even in poorly virilized cases at birth, male gender assignment may allow spontaneous puberty without signs of hypogonadotropic hypogonadism, and possibly fertility. Patients with SF-1 mutations are at increased risk for malignant germ cell tumors. In case of preserved gonads, early orchidopexy and germ cell tumor screening is warranted. The finding of premalignant and/or malignant changes should prompt gonadectomy or possibly irradiation.

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Introduction

Steroidogenic factor 1 (SF-1), encoded by the NR5A1 gene, is known to be involved in adrenal and gonadal development and differentiation. In addition, in both males and females, SF-1 is a key promoter of gonadal hormone production, by regulating the transcription of several genes involved in steroidogenesis (1–3). SF-1 mainly acts through direct binding to the promoter regions of its target genes, however, indirect effects have also been studied (1, 2, 4).

The clinical presentation of patients affected by NR5A1 mutations is very diverse, ranging in 46,XY individuals from phenotypically females with complete gonadal dysgenesis with or without adrenal failure (5–10) to males with penoscrotal hypospadias (8, 11, 12) or bilateral anorchia (13). In 46,XX women, NR5A1 mutations have been associated with premature ovarian failure (3). The clinical and genetic characteristics of these patients have been reviewed elsewhere (14–16).

The observed Sf-1 expression in the ventromedial hypothalamus and gonadotrophs and impaired gonadotrophin production in Sf-1 knockout mice suggest an additional role for Sf-1 in the normal regulation of puberty, reproduction, and metabolism (14, 17). In line
with this hypothesis, pituitary-specific SF-1 knockout mice develop hypogonadotropic hypogonadism (18). Since most of the initially reported 46,XY patients, on the basis of absent or poor virilization at birth, were sex-assigned female and received gonadectomy at a young age, little is known about the natural course of puberty in 46,XY patients with NR5A1 mutations. In addition, the risk for the development of germ cell tumors in older individuals remains an important but unexplored issue.

In this study, we present detailed clinical, hormonal, and histopathological investigations at the onset of puberty in two patients with NR5A1 haploinsufficiency, and with a predominantly female phenotype at birth. One of them was reassigned male at the age of 2 years after the finding of a palpable labioscrotal gonad and his testis function has been followed through puberty.

Material and methods

Hormonal investigations

Testosterone random values were obtained by coat-a-count RIA (Siemens, Los Angeles, CA, USA) (case 1) and liquid chromatography/tandem mass spectrometry (UPLC Waters quattro premier, Waters Corporation, Milford, MA, USA) (case 2). LH and FSH random values were obtained by electrochemoluminescence assay (Roche Diagnostics E170 Modular). Inhibin B (Inhibin B, Oxford Bio-Innovation Ltd, Oxford, UK) and AMH (Roche Diagnostics E170 Modular) were obtained by electrochemoluminescence assay. Inhibin B and AMH were measured by ELISA. Human chorionic gonadotropin (HCG) and ACTH tests were performed according to our local protocols. Values for testosterone were obtained at baseline and 60 min after i.v. injection of Synacthen (Novartis Pharma), 250 μg.

Gene analyses

Multiplex ligation-dependent probe amplification (MLPA) An in-house-designed synthetic probe set for MLPA was used to detect single exon deletions/duplications on the NR5A1 gene (19). MLPA reactions were performed by the reagents and the recommendations of www.eje-online.org. MLP A was used to detect single exon deletions/duplications (MLPA) on the basis of absent or poor virilization at birth, were sex-assigned female and received gonadectomy at a young age, little is known about the natural course of puberty in 46,XY patients with NR5A1 mutations. In addition, the risk for the development of germ cell tumors in older individuals remains an important but unexplored issue.

In this study, we present detailed clinical, hormonal, and histopathological investigations at the onset of puberty in two patients with NR5A1 haploinsufficiency, and with a predominantly female phenotype at birth. One of them was reassigned male at the age of 2 years after the finding of a palpable labioscrotal gonad and his testis function has been followed through puberty.

Table 1 Summary of primers used for sequence analysis of the NR5A1 gene.

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tbody>
<tr>
<td>NR5A1SQE0203</td>
<td>5’-gggacacagaggggtac-3’</td>
<td>-tcccttgagcag-3’</td>
</tr>
<tr>
<td>NR5A1SQE04-1</td>
<td>5’-gttcttgagcag-3’</td>
<td>-gggagctggg-3’</td>
</tr>
<tr>
<td>NR5A1SQE04-2</td>
<td>5’-ccgagggagag-3’</td>
<td>-gggacagag-3’</td>
</tr>
<tr>
<td>NR5A1SQE06</td>
<td>5’-cccacagtgcagtcac-3’</td>
<td>-ccgagggagag-3’</td>
</tr>
<tr>
<td>NR5A1SQE07</td>
<td>5’-atgccagcttttggg-3’</td>
<td>-gggagctggg-3’</td>
</tr>
</tbody>
</table>

Sequencing DNA was extracted from peripheral blood cells using standard procedures. The coding exons and exon/intron boundaries (exons 2–7) were amplified, using the primers summarized in Tables 1 and 2. All PCRs were performed by TaKaRa LA Taq polymerase. Subsequently, the fragments obtained were analyzed by direct sequence analysis on an ABI 3730XL DNA sequencer. Data analysis was carried out using Seqscape software (Applied Biosystems). The sequence variant was confirmed by a second experiment, including PCR, on the same DNA sample.

Pathological studies

Gonadal tissue was fixed in 10% formalin for 24 h. Slices of 3 μm thickness were prepared. Experimental conditions for immunohistochemistry are summarized in Tables 1 and 2. Staining was performed by DAB/H2O2 (OCT3/4 (POU5A1), SOX9, FOXL2, and SF-1 (NR5A1)) or New Fuchsin/Sodium nitrite Naphtol ASMX phosphate (TSPY and AMH), and counterstaining with hematoxylin.

Ethics

Informed consent to publish results was obtained from both patients and their parents.

Results

Case 1 is a girl from consanguineous parents, who first came to medical attention at the age of 13 years, because of progressive virilization in the past year (facial hair growth, deepening of the voice, and muscular body) and lack of breast development. Her younger sister and brother are healthy. Discrete clitoromegaly had been noticed at birth but was judged insufficient to prompt medical investigations. At presentation, she had pubic and axillary hair but no breast development (Tanner B1P3A2), clitoral length was 40 mm. On questionnaires on gender role behavior and gender
identity (21) she obtained scores in female ranges indicating that in behavior and identity she did not differ from other girls of her age. Clinical data, baseline hormonal investigations and values after HCG and ACTH tests are summarized in Table 3 and indicate, in spite of her initial female phenotype, impaired Sertoli cell but good Leydig cell function. Karyotype was 46,XY. By MLP A analysis, a heterozygous partial deletion (NCBI36/hg18 Chr9:g.(126306276-126307705)-(126303229-126302828)del), comprising exons 2 and 3, that are the first two coding exons, was detected (19). At laparoscopy, the left and right gonads were found in the inguinal and abdominal position, respectively, and bilateral gonadectomy was performed. There was no uterus. Pathological examination revealed bilateral testes with pronounced Leydig cell hyperplasia, normal seminiferous tubules, normal germ cell number in the left (inguinal) gonad, with incomplete spermatogenesis, and scarce germ cells in the right (abdominal) gonad (Fig. 1). On both sides, small, well-defined

Table 2 Experimental conditions for the pathological studies.

<table>
<thead>
<tr>
<th>Primary AB</th>
<th>Supplied by</th>
<th>Dilution</th>
<th>Pretreatment</th>
<th>AG retrieval</th>
<th>Incubation time</th>
<th>Secondary AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT3/4</td>
<td>Santa Cruz Biotechnology, Santa Cruz, CA, USA</td>
<td>1/1000</td>
<td>3% H2O2 for 5’</td>
<td>pH 6, biotin blocking</td>
<td>2 h, room temperature</td>
<td>HAG, biotin labeled</td>
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<tr>
<td>TSPY</td>
<td>Kindly provided by C C Lau</td>
<td>1/3000</td>
<td>–</td>
<td>pH 6</td>
<td>Overnight, 4 °C</td>
<td>SAR, biotin labeled</td>
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<tr>
<td>SOX9</td>
<td>AF3075, R&amp;D Systems, Wiesbaden, Germany</td>
<td>1/250</td>
<td>3% H2O2 for 5’</td>
<td>pH 6, biotin blocking</td>
<td>2 h, room temperature</td>
<td>HAG, biotin labeled</td>
</tr>
<tr>
<td>FOXL2</td>
<td>Kindly provided by R A Veitia, M Fellous, F Jaubert</td>
<td>1/350</td>
<td>3% H2O2 for 5’</td>
<td>pH 6, biotin blocking</td>
<td>Overnight, 4 °C</td>
<td>SAR, biotin labeled</td>
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<tr>
<td>AMH</td>
<td>Kindly provided by A Themmen</td>
<td>1/200</td>
<td>3% H2O2 for 5’</td>
<td>pH 6, biotin blocking</td>
<td>Overnight, 4 °C</td>
<td>RAM, biotin labeled</td>
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<tr>
<td>SF-1</td>
<td>PP-N1665-00, R&amp;D Systems</td>
<td>1/100</td>
<td>3% H2O2 for 5’</td>
<td>pH 6, biotin blocking</td>
<td>Overnight, 4 °C</td>
<td>RAM, biotin labeled</td>
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AB, antibody; AG, antigen; HAG, horse anti-goat; SAR, swine anti-rabbit; RAM, rabbit anti-mouse.

Table 3 Summary of clinical, hormonal, genetic, and histopathological data in patients 1 and 2.

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
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<tr>
<td><strong>Clinical data</strong></td>
<td><strong>Pathological data</strong></td>
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<tr>
<td><strong>Phenotype at birth</strong></td>
<td><strong>Sample</strong></td>
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<tr>
<td><strong>Uterus</strong></td>
<td><strong>Location</strong></td>
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<tr>
<td><strong>Sex</strong></td>
<td><strong>Differentiation</strong></td>
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<td><strong>Gender identity</strong></td>
<td><strong>Histology</strong></td>
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<tr>
<td><strong>Pubertal development</strong></td>
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<td>13 Years: marked facial hair growth, Adam's apple, deepening of the voice, muscular body, clitoris length 40 mm. Pubertal stage B1P3A2</td>
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SPL, stretched penile length; NA, not available; T, testosterone obtained by RIA after solvent extraction and chromatography; T*, testosterone obtained by LC MS/MS; HCG, human chorionic gonadotrophin normal values (males); T (pubertal, RIA), 3.01–29.95 nmol/l; T* (pubertal, LC MS/MS), 11.13–34.84 nmol/l; Inhibin B (Tanner 1), 35–182 ng/l; AMH (Tanner 1), 395–1397 pmol/l; LH 1–9 U/l; FSH 1–12 U/l.

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testicular regions displayed OCT3/4 positive, TSPY-positive germ cells on the basal membrane, as well as positivity for the c-KIT ligand stem cell factor (SCF), thus fulfilling the criteria of a developing carcinoma in situ (CIS) lesion of the testis, as described in (22) and (23). No OCT3/4 positivity was found in the rest of the gonads, indicating maturation of the germ cells beyond the gonocyte, i.e. prespermatogonial stage in these regions. In the nuclei of both Leydig and Sertoli cells, a homogenous SF-1 staining pattern was noticed, additionally, in the Leydig cell cytoplasm, but not in Sertoli cells, a granular staining pattern was observed. For comparison, SF-1 expression was examined in two testes of normal male controls and three human fetal testes, obtained after spontaneous abortion in the 16th week of gestation, and after stillbirths in the 30th and 40th weeks of gestation. In adult testes, the SF-1 staining pattern in Leydig and Sertoli cells was similar to that in patient 1. However, in the three fetal testes, Leydig cells only revealed the nuclear, but not the cytoplasmic staining pattern (Fig. 2). After gonadectomy, hormonal replacement therapy was initiated, which induced normal breast development. ACTH test, performed at 15 years was normal.

Figure 1 (A) Case 1, left gonad. Pubertal testis with lumen (L) formation, Leydig cell hyperplasia (LCH), normal germ cells (arrowheads) with incomplete spermatogenesis (SG). HE staining, 100×. (B) Same gonad. Abundant TSPY-positive germ cells (stained red), incomplete spermatogenesis, with spermatogonia (SG) and primary spermatocytes (SC I). TSPY staining, 200×. (C) Same gonad. OCT3/4-positive (arrowheads) cells (stained brown) on the basal lamina are indicative of a developing CIS lesion. OCT3/4 staining, 200×. (D) Same gonad. SCF positivity (arrowheads) in the same area as the OCT3/4-positive cells. SCF staining, 100×. (E) Case 1, right gonad. Pubertal testis with predominantly Sertoli cell only (SCO) tubules and Leydig cell hyperplasia (LCH). HE staining, 100×. (F) Same gonad. Scarce TSPY-positive (red) spermatogonia (arrowheads) are present in well-circumscribed areas of the gonad. Adjacent tubules are devoid of germ cells. TSPY staining, 100×. (G) Same gonad. OCT3/4-positive (arrowheads) cells (stained brown) on the basal lamina are indicative of a developing CIS lesion. OCT3/4 staining, 200×. (H) Same gonad. SCF positivity (arrowheads) in the same area as the OCT3/4-positive cells. SCF staining, 200×.

Figure 2 (A and B) Case 1, left and right gonad. Homogenous nuclear SF-1 staining in Sertoli cells (arrowheads). In Leydig cells (arrows), SF-1 staining is characterized by a homogenous nuclear pattern and a granular cytoplasmic staining pattern. SF-1 staining, 400×. (C) The same staining pattern is found in Sertoli cells (arrowheads) and Leydig cells (arrows) of an adult male control. SF-1 staining, 400×. (D–F) In fetal Leydig cells (arrows), as well as in Sertoli cells (arrowheads), only a nuclear SF-1 staining pattern is found, whereas the typical granular cytoplasmic SF-1 staining pattern as it is found in adult Leydig cells is absent. D human fetal testis, 16 weeks gestational age, E human fetal testis, 30 weeks gestational age, and F human fetal testis, 40 weeks gestational age. SF-1 staining, 400×.
Case 2 is the fourth child from unrelated parents. At birth, enlarged labia with posterior fusion and clitoral hypertrophy (not measured) were found, somewhat later; a left labial gonad was palpated. Karyotype was 46,XY. A tentative diagnosis of partial androgen insensitivity syndrome was made without further investigations, and a female sex was assigned. At the age of two, the child was referred to the pediatric urologist of our center to perform vaginoplasty. Physical examination at that age revealed clitoris hypertrophy (20 mm), bilateral gonads palpable in partially fused labioscrotal folds, and a single perineal urogenital opening. On the basis of the findings at physical examination, and after hormonal evaluation (Table 3) and psychological counseling, the child was reassigned male, and multistage hypospadias correction was performed. Since his penis remained very short, the child and his parents were explained that phalloplastic surgery would be necessary in young adulthood. At the age of 12 years, gonads were found to be in the inguinal position, bilateral orchidopexy was performed and biopsies were taken bilaterally. Histological analysis revealed normal prepubertal testicular architecture, with somewhat irregular distribution of germ cells, resembling prespermatogonia. In line with this, OCT3/4 staining was negative, indicating absence of germ cell maturation delay or CIS (Fig. 3A–D). Spontaneous initiation of puberty was noticed at 13 4/12 years, stretched penile length at that moment was 21 mm. This was followed by a progressive virilization of his external genitalia and a normal growth spurt, accompanied by an appropriate rise in IGF1 concentrations and a near-final height in accordance with midparental height. Maturation of secondary sexual characteristics was mainly recorded in the first 24 months of puberty, with little evolution thereafter. Clinical and hormonal data throughout puberty are represented in Table 3 and graphically depicted in Fig. 4. No sperm was found in an ejaculate sample, collected at the age of 15 years, concordant with the testicular volumes (12 and 10 ml) at that time. At the last visit (at 16 10/12 years), pubertal development was nearly complete (Tanner stage G4P4A2), left and right testicular volumes were 12 and 10 ml, respectively, stretched penile length was 58 mm, penile width was judged normal, there is no indication for phalloplastic surgery. Although gender identity was not formally tested, gender dysphoric feelings were never reported by the patient or by his parents, even when specifically asked for, and he has...
a heterosexual orientation. Bilateral testicular biopsy specimens, taken at the age of 15 10/12 years, are stored for eventual testicular semen extraction in the future. Genetic analysis revealed a heterozygous point mutation in exon 2 of the NR5A1 gene, replacing the normal tyrosine by a premature stop codon at this position (c.9T>A, p.Tyr3X) (Fig. 5). ACTH test, performed at 14 years was normal. The mutation was not demonstrated in the patient’s mother, paternal DNA was not examined.

Discussion

The role of SF-1 as a key regulator of steroidogenesis is well established (14, 24). Many 46,XY patients with NR5A1 mutations are born with severely undervirilized genitalia, although the clinical presentation may vary considerably (reviewed in (14–16)). In addition, Sf-1 acts as an inducer of gonadotrophin expression, and pituitary-specific Sf-1 knockout mice develop hypogonadotropic hypogonadism and sexual infantilism, due to the absent expression of LH and FSH (18, 24, 25). In contrast to this, hypergonadotropic hypogonadism is documented in many patients with NR5A1 mutations (6–9), suggesting functional differences of SF-1 in humans compared with mice with regard to gonadotropin expression and puberty.

Since most of the initially reported 46,XY patients, on the basis of absent or poor virilization at birth, were sex-assigned female and received gonadectomy at a young age, little is known about the natural course of puberty in older individuals with NR5A1 mutations. Partial hypogonadotropic hypogonadism, necessitating testosterone supplementation during puberty has been documented in one male patient with penoscrotal hypospadias and a L437Q mutation in the NR5A1 ligand-binding domain (8). Recently, normal pubertal progression, with low Inhibin B but normal LH and FSH concentrations has been reported in two adolescents with a milder phenotype (12). In this study, we report on spontaneous and advanced androgenization in two adolescents who were poorly virilized at birth and sex-assigned female, with reassignment to male in one patient at the age of 2 years. Neither of the patients present with hypogonadotropic hypogonadism, although puberty starts somewhat late in case 2 (at 13 years). Testicular architecture is well preserved in both patients, but hormonal work up clearly suggests gonadal dysgenesis in case 1 (increased FSH, low Inhibin B), whereas this is less obvious in case 2 (discrete rise in FSH, normal values for AMH and Inhibin B).

In both patients, the severe clinical phenotype at birth is in line with the detected NR5A1 mutations. In case 1, since exon 2 is the first coding exon, there is no protein production. If the ATG at codon 88 within exon 4 is used as alternative starting ATG, the resulting protein would be missing the DNA-binding domain encoded by exons 2 and 3 and would be an N-terminally truncated protein. In case 2, the first ATG after codon 3 is the ATG at codon 78 at the end of exon 3. Even in this

![Figure 5 Case 2: NR5A1 sequencing results in the patient and his mother, who is no carrier of the mutation. Electropherograms of part of amplicon NR5A1SQE0203 from the index patient and the mother, obtained with a forward sequence primer (F) or a reverse sequence primer (R). The arrow points to the sequence variant c.9T>A, p.Tyr3X which is present in a heterozygous pattern in DNA of the patient.](www.eje-online.org)
case, if a protein is synthesized, it would be missing the DNA-binding domain.

The poorly virilized neonatal phenotype of 46,XY individuals with NR5A1 mutations suggests that the in utero virilization of external genitalia requires much higher testosterone concentrations than those necessary for Wolffian duct stabilization and differentiation, or alternatively, that 5α-reductase activity (which converts testosterone to its more active metabolite dihydrotestosterone) is influenced by SF-1, specifically during intrauterine development, which has not been demonstrated so far (24). The first hypothesis is in line with the finding of advanced Wolffian duct development in some patients with complete androgen insensitivity syndrome (26). Our data, as well as others’ (9, 12) however, suggest a remarkable restoration of Leydig cell function during puberty. In our gonadal samples, the immunohistochemical expression pattern of SF-1 in adult Leydig cells was different from the expression pattern in fetal Leydig cells, which possibly reflects a functional difference. It has been suggested that fetal Leydig cells represent a different population than adult Leydig cells (27, 28). SF-1-mediated induction of steroidogenic enzymes has not been studied in detail in these two different populations, and it is conceivable that in vivo steroidogenesis by adult Leydig cells is less dependent on SF-1 compared with steroidogenesis by fetal Leydig cells. This hypothesis is supported by in vitro studies of MAML1 function: MAML1 plays a supportive role in testosterone production and has been shown to be a target for SF-1 (29, 30). However, the murine Maml1 homolog is only transiently expressed during the critical period of mouse gonadal development and differentiation. Human MAML1 mutations have been associated with a clinical phenotype of isolated severe hypospadias, and thus, in males. SF-1 mediated MAML1 action is believed to be supportive for fetal but not adult testosterone production and virilization of external genitalia. Alternatively, steroidogenesis in patients with NR5A1 mutations may be compromised to a higher degree during fetal life due to impaired Sertoli cell function. Indeed, a shift of HSD17B3 activity from Sertoli cells in infantile testes to Leydig cells in pubertal testes has been postulated (31). Thus, the end product of steroidogenesis in fetal Leydig cells is androstenedione, which is apparently effective only after conversion to testosterone by Sertoli cell mediated HSD17B3 activity. Partially compromised Sertoli cell function in NR5A1 mutations is suggested by the finding of low AMH and Inhibin B concentrations, and/or increased FSH. At puberty, HSD17B3 activity is found in the adult Leydig cell type, which enables direct testosterone production, independent of Sertoli cell function. Finally, several lines of evidence suggest that in the adult testis, the regulatory role of SF-1 on steroidogenesis can be functionally replaced by liver receptor homolog 1 (LRH-1 or NR5A2). LRH-1 is another member of the NR5A nuclear receptor family, which is abundantly expressed in adult but not in fetal Leydig cells (32–35), explaining the unexpected virilization during puberty in our, as well as in other patients (9). Future research focusing on the dimorphic effects of NR5A1 during intrauterine and pubertal development to solve these issues would be of high interest.

Since poor virilization at birth does not seem to exclude adequate male pubertal development, this needs to be taken into account when considering definitive sex assignment. With regard to gender identity, we feel that general conclusions cannot be drawn from a favorable outcome in two patients, but gender identity in patients with NR5A1 mutations seems at least to be flexible and not necessarily concordant with the degree of external virilization at birth. While considering male sex assignment, one should take into account the later possibilities for fertility and for the development of malignancy of the germ cells (36). Although prespermatogonia were abundantly present in a prepubertal testis biopsy, no sperm could be detected in the ejaculate of case 2 at the age of 15 years. However, gonadal tissue was cryopreserved in view of possible testicular semen extraction in the future. Hitherto, invasive type II germ cell tumors, as are seen in various groups of patients with 46,XY disorders of sex development (reviewed in (37, 38)) have not been reported in patients with NR5A1 mutations. A developing CIS, according to the criteria outlined in an earlier study (22), was clearly present bilaterally in case 1, which is known to progress to invasiveness if untreated. The prolonged non-scrotal location of the gonads has been recognized as an additional risk factor for tumor development (39). However, it is expected that the risk in patients with NR5A1 mutations is intrinsically increased due to testicular dysgenesis. Therefore, if a decision is made to rear patients in the male gender, this needs to be accompanied by immediate orchidopexy in the case of non-scrotal gonads, and rigorous follow-up (by self-examination, clinical follow-up and regular ultrasound) of testis position, growth and consistency, especially during puberty and thereafter.

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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