CLINICAL STUDY

Testosterone production during puberty in two 46,XY patients with disorders of sex development and novel NR5A1 (SF-1) mutations

Sally Tantawy, Lin Lin1, Ilker Akkurt2, Guntram Borck3, Dietrich Klingmüller4, Berthold P Hauffa5, Heiko Krude, Heike Biebermann, John C Achermann1 and Birgit Köhler

Department of Pediatric Endocrinology, University Children’s Hospital, Charité, Humboldt University, Augustenburger Platz 1, 13353 Berlin, Germany, 1UCL Institute of Child Health, University College London, London, UK, 2Children’s Hospital Altona, Hamburg, Germany, 3Institute of Human Genetics, University of Ulm, Ulm, Germany, 4Institute for Clinical Chemistry and Pharmacology, University of Bonn, Bonn, Germany and 5Department of Pediatric Endocrinology and Diabetes, University Children’s Hospital, University Duisburg-Essen, Essen, Germany

(Correspondence should be addressed to B Köhler; Email: birgit.koehler@charite.de)

Abstract

Background: Steroidogenic factor 1 (SF-1, NR5A1) is a key transcriptional regulator of many genes involved in the hypothalamic–pituitary–gonadal axis and mutations in NR5A1 can result in 46,XY disorders of sex development (DSD). Patients with this condition typically present with ambiguous genitalia, partial gonadal dysgenesis, and absent/rudimentary Müllerian structures. In these cases, testosterone is usually low in early infancy, indicating significantly impaired androgen synthesis. Further, Sertoli cell dysfunction is seen (low inhibin B, anti-Müllerian hormone). However, gonadal function at puberty in patients with NR5A1 mutations is unknown.

Subjects and methods: Clinical assessment, endocrine evaluation, and genetic analysis were performed in one female and one male with 46,XY DSD who showed spontaneous virilization during puberty. The female patient presented at adolescence with clitoral hypertrophy, whereas the male patient presented at birth with severe hypospadias and entered puberty spontaneously. Molecular analysis of NR5A1 was performed followed by in vitro functional analysis of the two novel mutations detected.

Results: Testosterone levels were normal during puberty in both patients. Analysis of NR5A1 revealed two novel heterozygous missense mutations in the ligand-binding domain of SF-1 (patient 1: p.L376F; patient 2: p.G328V). The mutant proteins showed reduced transactivation of the CYP11A promoter in vitro.

Conclusion: Patients with 46,XY DSD and NR5A1 mutations can produce sufficient testosterone for spontaneous virilization during puberty. Phenotypic females (46,XY) with NR5A1 mutations can present with clitoromegaly at puberty, a phenotype similar to other partial defects of androgen synthesis or action. Testosterone production in 46,XY males with NR5A1 mutations can be sufficient for virilization at puberty. As progressive gonadal dysgenesis is likely, gonadal function should be monitored in adolescence and adulthood, and early sperm cryopreservation considered in male patients if possible.

European Journal of Endocrinology 167 125–130

Introduction

Steroidogenic factor 1 (SF-1, NR5A1), a member of the nuclear receptor superfamily, is a key transcriptional regulator of many genes involved in the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal axes (e.g. inhibin α, DAX1 (NR0B1), STAR (STAR), CYP11A (CYP11A1), CYP17A1, HSD3B2, CYP19A1, MIS (AMH), oxytocin, and SOX9) (1). SF-1 is highly expressed in steroidogenic tissues from very early stages of development, including the developing gonad and adrenal, and continues to be expressed in these tissues into adult life (2). SF-1 is also expressed in the ventromedial hypothalamic nucleus and pituitary gonadotropes (3, 4, 5). Consistent with this central role in reproductive and adrenal development, XY Sf-1 knockout mice (Sf-1<sup>−/−</sup>) lack adrenal glands and show male to female ‘sex reversal’ due to complete gonadal dysgenesis (6). Furthermore, male gonad-specific Sf-1<sup>−/−</sup> mice display hypoplastic testes and internal genitalia, undescended testes, and infertility (7). SF-1 interacts in vitro with several other major factors playing a role in gonadal determination and differentiation (e.g. SRY, GATA4, and SOX9) (8, 9, 10).

To date, about 50 different NR5A1 mutations have been reported in humans with disorders of sex development (DSD). NR5A1 mutations were first described in two 46,XY patients with complete gonadal dysgenesis and primary adrenal failure (11, 12) and in one 46,XX female with primary adrenal failure (13).
More recently, however, many more heterozygous
NR5A1 mutations have been identified in patients
with 46,XY DSD without adrenal insufficiency. The
majority of these 46,XY DSD patients without adrenal
failure have a phenotype of ambiguous genitalia at
birth, partial gonadal dysgenesis, and absent or
rudimentary Müllerian structures (14, 15, 16). The
frequency of NR5A1 mutations in patients with this
phenotype has been estimated to be about 15% (17,
18). Furthermore, NR5A1 mutations have also been
identified in 46,XY patients with hypospadias and
cryptorchidism, which is likely to be caused by a milder
form of partial gonadal dysgenesis (19, 20), as well as
in association with vanishing testis syndrome, micro-
penis, or infertility (21, 22, 23). In addition, SF-1 plays
a role in ovarian development and function and
mutations have been reported in women (46,XX) with
primary ovarian insufficiency and premature ovarian
failure (24). NR5A1 mutations rarely cause isolated
adrenal insufficiency (25). Therefore, it is currently
thought that disruption of SF-1 has a more significant
effect on human gonadal development and function
compared with human adrenal development and
function (25).

In most published cases of 46,XY DSD due to NR5A1
mutations, testosterone has been low indicating signi-
ficantly impaired androgen synthesis. Sertoli cell
hypofunction has been suggested because of low levels
of inhibin B and anti-Müllerian hormone (AMH) and
elevated FSH. However, normal testosterone levels at
birth have been reported in one patient with 46,XY DSD
and low normal testosterone levels during puberty have
been described in another patient (23, 26). We report
two novel NR5A1 mutations in two patients with 46,XY
DSD who demonstrate normal male testosterone
production during puberty.

**Subjects**

Written informed consent for genetic analysis was
obtained according to institutional ethical guidelines
from the parents of patient 1 and from patient 2.

**Patient 1**

Patient 1 is a female who presented at puberty with
clitoral hypotrophy. She was born to nonconsanguineous
German Caucasian parents. The clinical presenta-
tion, endocrine and laparoscopic findings, and
gonadal histology are summarized in Table 1. Psycho-
logical evaluation of the patient showed female gender
identity. At 14 6/12 years, bilateral gonadectomy was
performed with the patient’s consent and estrogen
substitution was initiated. Mutations of 5-α reductase
type 2 (SRD5A2), 17-β hydroxysteroid dehydrogenase
type 3 (HSD17B3), and androgen receptor (AR) genes
were excluded. Basal cortisol was normal at puberty.

**Patient 2**

Patient 2 is a 28-year-old male who first presented at
birth with severe penoscrotal hypospadias, hypoplastic
phallus, and small inguinal testes. He was born to
nonconsanguineous German Caucasian parents. In
early infancy, a two-step surgical correction of hypo-
spadias was carried out. The clinical presentation,
testicular histology, results of laparoscopy, pubertal
development, hormones, and semen analysis of the
patient are summarized in Table 1. Mutations of the
SRD5A2, AR, and LH/choriogonadotropin receptor
(LHCGR) genes were excluded. Testosterone sub-
stitution was started at 28 years of age. Basal and
stimulated cortisol (Synacthen test) levels were normal
in adulthood.

**Methods**

**Molecular analysis of NR5A1 gene**

Molecular analysis of exons 2–7 of the gene encoding
SF-1 (NR5A1) was performed as described previously
(18). DNA mutation numbering is based on GenBank
reference DNA sequence NM_004959.4, with the A of
the ATG initiation codon designated +1 (www.hgvs.org/mutnomen).

**Functional characterization**

*In vitro* functional activities of the NR5A1 mutations on
the murine *Cyp11a* promoter was assessed by transient
gene expression assays in human embryonic kidney
TSA-201 cells as described previously (17).

**Results**

**Molecular genetic analysis and functional
colorization**

Analysis of the NR5A1 gene revealed a novel hetero-
zgyous missense mutation c.1126C>T (p.L376F,
g.127253372) in exon 6 in patient 1 and a novel
heterozygous missense mutation c.983G>T (p.G328V,
g.127255316) in exon 5 in patient 2. Both mutations
are located in the ligand-binding domain of SF-1
(Fig. 1). The p.L376F alteration is a *de novo* mutation
as neither parents of patient 1 harbored this change.
Only the mother of patient 2 was available for genetic
analysis and she did not harbor the mutation. The
mutated amino acids, leucine at position 376 and
glycine at position 328, are highly conserved in SF-1
orthologs (Fig. 1). The c.1126C>T and c.983G>T
variants are unlikely to be neutral nonpathological
polymorphisms as they are not present in the database
of single-nucleotide variants (dbSNP132) or the
1000 Genomes data (www.1000genomes.org/data).
Functional analysis of the mutants showed reduced activities to 12% (p.L376F) and 8% (p.G328V) of wild-type activity on the Cyp11a promoter (Fig. 2). No functional activity of the mutants on the AMH, TESCO, and INSL3 promoter was investigated as the two patients did not show either persistent uterine development as a sign of disturbed AMH secretion or severe gonadal dysgenesis as a result of impaired testes determination.

**Discussion**

We report two novel NR5A1 mutations (p.G328V and p.L376F) in two 46,XY DSD patients with normal male testosterone production at puberty. To date, knowledge about gonadal function at puberty in patients with 46,XY DSD and NR5A1 defects is very scarce, as most cases identified have undergone gonadectomy in early infancy. Only one 46,XY DSD patient with low normal testosterone production during puberty has been reported (26).

The first patient we describe (patient 1) is a 46,XY female who presented at puberty with clitoral hypertrophy, bilateral dysgenetic abdominal testes, no Müllerian structures, testicular histology: bilateral testicular tissue, no spermatogenesis, inhibin B < 10 pg/ml, steroid profile: normal.

Table 1

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Clinical presentation</th>
<th>Testosterone (ng/ml)</th>
<th>FSH (IU/l)</th>
<th>LH (IU/l)</th>
<th>Other investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>B1, P4, A1 Clitoromegaly</td>
<td>2.1</td>
<td>35.9</td>
<td>2.3</td>
<td>Karyotype: 46,XY Laparoscopy: abdominal testes, no Müllerian structures Testicular histology: bilateral testicular tissue, no spermatogenesis Inhibin B: &lt; 10 pg/ml Steroid profile: normal</td>
</tr>
<tr>
<td>3.5</td>
<td>Severe penoscrotal hypospadias Small inguinal testes Penile length 1 cm Penile length 2.5 cm</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Karyotype: 46,XY</td>
</tr>
<tr>
<td>13.5</td>
<td>G2, P2, A1 Gynecomastia B2 (3 cm Ø) Penile length 4.7 cm Right testis 5 ml, left testis 4 ml</td>
<td>3.9 → 6.5 (after HCG 5000 IU/m²)</td>
<td>25.9</td>
<td>3.4</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>G4, P5, A3 Gynecomastia B4–5 (9 cm Ø) Penile length 7.1 cm Right testis 13 ml, left testis 15 ml</td>
<td>2.6</td>
<td>35.4</td>
<td>7.0</td>
<td>–</td>
</tr>
<tr>
<td>19</td>
<td>Penile length 6 cm Right testis 6 ml, left testis 6 ml</td>
<td>3.3</td>
<td>37.9</td>
<td>10.9</td>
<td>Surgery for gynecomastia</td>
</tr>
<tr>
<td>28</td>
<td>Right testis 6 ml, left testis 6 ml</td>
<td>2.6</td>
<td>58.7</td>
<td>14.0</td>
<td>Inhibin B: undetectable Spermiogram: azoospermia</td>
</tr>
</tbody>
</table>

NA, not available; HCG, human chorionic gonadotrophin.

www.eje-online.org
of a small Muellerian rest. Neither of the patients we impaired AMH production is suggested by the presence of duct regression in patient 1. In patient 2, partially adequate AMH production and subsequent Muellerian ionic/fetal Sertoli cell function was sufficient for embryonic and fetal development. In contrast, embryonic cell hypofunction.

inhibin B concentrations, reflecting Leydig and Sertoli cell function with age(23). The mutated leucine at position 376 and glycine at position 328 are conserved in SF-1 orthologs.

during puberty in patients with NR5A1 mutations can result in significant testosterone production in some cases.

Patient 2, a 46,XY male born with ambiguous genitalia, had sufficient testosterone synthesis at puberty for spontaneous virilization and male pubertal development to occur. However, toward the end of puberty, he developed progressive gonadal failure affecting both Leydig and Sertoli cells, with evidence of deteriorating function through adulthood. At 28 years, his testosterone was very low, inhibin B was undetectable, and azoospermia was present. The delayed orchidopexy may have contributed to the azoospermia but is unlikely to have been causative. Previously, we have reported NR5A1 mutations in about 10% of patients with a similar phenotype of complex hypospadias at birth (severe hypospadias and/or hypoplastic phallus and cryptorchidism) (19, 20). However, only one other patient with complex hypospadias at birth and testosterone production during puberty has been reported: Philibert et al. (26) described a 46,XY boy with hypospadias and micropenis at birth who was found to harbor a heterozygous NR5A1 mutation (p.R281P) in the ligand-binding domain of the protein. He entered puberty spontaneously with a low-normal rise of testosterone but had elevated FSH and decreased inhibin B concentrations, reflecting Leydig and Sertoli cell hypofunction.

Both patients reported in our study showed various degrees of underandrogenization at birth revealing that Leydig cell function had already been disturbed during embryonic and fetal development. In contrast, embryonic/fetal Sertoli cell function was sufficient for adequate AMH production and subsequent Mullerian duct regression in patient 1. In patient 2, partially impaired AMH production is suggested by the presence of a small Mullerian rest. Neither of the patients we describe showed any signs or symptoms of adrenal insufficiency as is the case with the majority of patients with heterozygous NR5A1 mutations published previously. Although detailed tests of adrenal function are not available for patient 1, we would currently recommend more comprehensive adrenal testing (e.g. cortisol response to Synacthen, and basal ACTH) and an ongoing awareness of the potential risk of adrenal failure in these patients. Further studies are required to systematically address the risk of adrenal dysfunction in such individuals in the long term.

In a recent study on male infertility, NR5A1 mutations were described in seven out of 199 (3.5%) males with moderate/severe oligospermia or azoospermia (23). All mutations were missense mutations in the hinge region or proximal ligand-binding domain of the protein. Notably, normal testosterone and low normal/undetectable inhibin B levels were found in two patients under 30 years of age while the older patients had low to undetectable testosterone and inhibin B levels. These observations suggest that infertile males with NR5A1 mutations are at risk of deterioration of gonadal function with age (23).

Our study and the studies of Philibert et al. and Bashamboo et al. show that patients with NR5A1 mutations can produce significant levels of testosterone in adolescence and can enter puberty spontaneously. However, progressive gonadal dysgenesis resulting in oligospermia or azoospermia is potentially likely in individuals where spermatogenesis has occurred. Therefore, we strongly recommend regular monitoring of gonadal function in adolescence and adulthood in

Figure 1 (A) Structure of the gene encoding SF-1 (NR5A1) showing different mutations: the two novel mutations reported in this study (p.L376F and p.G328V); mutations identified in patients with male infertility (p.G123A, p.P129L, p.P131L, p.R191C, p.G212S, and p.D238N) (23); mutation of a 46,XY DSD patient with hypospadias and low-normal testosterone production (p.R281P) (26). (B) The mutated leucine at position 376 and glycine at position 328 are conserved in SF-1 orthologs.

Figure 2 Effect of the SF-1 mutants on transcriptional activity of the minimal promoter of Cyp11a in TSA-201 cells. A previous loss of function change in SF-1/NR5A1 (p.G35E) was used as a control for loss of activity. All values represent the means of three separate transfection experiments (± S.E.M.).
males with NR5A1 mutations. If sperms are detected, early cryopreservation could be undertaken with appropriate genetic counseling in an attempt to preserve future chances of fertility.

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.

Funding
This work was supported by the DAAD German Egyptian Long-term Scholarship (S Tantawy A1092239) and the Wellcome Trust Senior Fellow in Clinical Science (J C Achermann 079666).

References

Testosterone at puberty in 46,XY DSD with NR5A1 mutations


Received 2 November 2011
Revised version received 17 February 2012
Accepted 3 April 2012