Functional Characterization of Two Mutations Located in the Ligand Binding Domain in the SF1


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Abstract

Purpose: Since SF1 gene mutations located in the ligand binding domain are associated with a wide phenotypic spectrum in 46,XY subjects, the functional and structural characterization of these variations is of great interest. The aim of this study is to evaluate the clinical phenotype, hormonal pattern and molecular studies (genetic, functional data and protein structural analysis) in two non-related 46,XY index patients.

Methods: Clinical characteristics, genomic DNA sequencing analysis, protein prediction software study and protein structure analysis, and functional characterization of the mutations was carried out.

Results: Both index DSD patients showed a similar phenotype, however several affected members of Family 1 showed variable phenotypes. While in Family 1 a previously reported heterozygous missense point mutation (p.Arg313His), found in Family 2 a novel heterozygous missense point mutation (p.Ser303Arg) was detected. Both mutations were predicted to be as "probably damaging". The transcriptional activity of SF1 mutants p.Arg313His and p.Ser303Arg, studied using two different promoters in two cell lines, exhibited significant reductions of transactivation activity. Structural analysis showed differences between both mutants, such as changes in the flexibility of the receptor backbone and in the tertiary structure around the ligand and in the AF-2 domain.

Conclusions: One of these ligand binding domain mutations in SF1 showed phenotypic heterogeneity among family members, while both variations showed similarities in prepubertal phenotype, as well as in damage prediction and experimental decreases in transcriptional activity, but marked differences in structural consequence predictions. Finally the present study reinforces the concept of the wide variability in the clinical phenotype in affected 46,XY DSD patients.

Keywords: Steroidogenic factor-1; SF1; NR5A1 gene; LBD mutations; 46,XY DSD

Introduction

Steroidogenic factor-1 (SF1/AD4BP/FTZF1) plays a key role in the regulation of adrenal and reproductive organs differentiation. The SF1 gene (NR5A1) is an autosomal gene located on chromosome 9q33 and it is the member 1 of the nuclear receptor subfamily 5, group A [1]. It expands over 30 kb of genomic DNA and it is comprised of one non-coding exon (I) followed by six coding exons (II to VII).

The protein consists of 461 amino acids that include a DNA-binding domain (DBD) containing two zinc fingers, a hinge region containing a first functional activation domain (AF-1), a ligand-binding domain (LBD) of 12 helices (H1-H12) that includes a second functional activation domain (AF-2), and an accessory region [2]. It is expressed in undifferentiated gonads even before SRY and it is necessary for testis determination and differentiation. SF1 protein is highly expressed in steroidogenic tissues, such as gonads, adrenals, and placenta, and regulates almost all the enzymes related to this process. It is also expressed in the ventromedial hypothalamic nucleus and pituitary gonadotropes with relevant physiological roles in the central nervous system [3].

Homozygous null mice (Nr5a1−/−) have adrenal and gonadal agenesis, persistent Müllerian structures in XY karyotype, partial hyponadotropic hypogonadism, and other features such as hypospleenism, abnormalities of the ventromedial hypothalamus, and late-onset obesity [4,5].

In humans, mutations in NR5A1 were reported to cause both 46,XY and 46,XX gonadal dysgenesis with or without adrenal failure [6-8]. Variable loss of SF1 function is associated with a wide phenotypic spectrum indicating that SF1 dosage is critical. In the approximately 81 patients so far described with impairment to the protein function mutations in the NR5A1 gene the clinical phenotype is largely variable including, female genitalia, mild clitoromegaly, isolated hypospadias, anorchia and even normal male genitalia with infertility [6,9]. In 46,XX affected female patients, mutations in NR5A1 are associated with different phenotypes including primary and secondary amenorrhea, premature menopause and decreased ovarian reserve, but preserved fertility [7,8,10].

We are reporting the clinical phenotype, hormonal pattern and molecular studies (genetic, functional data and protein structural analysis) in two non-related 46,XY, DSD index patients and their affected family members. A novel heterozygous NR5A1 gene mutation, c.909G>A (p.Ser303Arg), and a previously described [8,10] heterozygous NR5A1 gene mutation, c.938G>A (p.Arg313His), both located in exon 5, in the

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highly conserved helix 5 (H5) of the ligand binding domain of the protein (LBD), are presented. Functional studies and protein structure analyses shed light into the reasons sustaining the pathogenicity of the mutations.

**Subjects and Methods**

**Clinical material**

Two families with an index case presenting with a 46,XY DSD were evaluated. Four generations of Family 1 and two generations of Family 2 are depicted in the family tree of Figure 1.

The clinical and biochemical findings in the affected members of the first family kindred have been previously reported [10]. Family 2 has not been previously reported.

This study was approved by the Ethics Committee of the Garrahan Pediatric Hospital. Written informed consent for the study was obtained from all adult patients or patients’ parents or tutors.

**Mutation analysis of the NR5A1 gene**

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures. The coding exons (exons 2-7) and flanking intronic regions of the NR5A1 gene were amplified by PCR using specific primers gently provided by Dr. J. C. Achermann, University College London, UK. The PCR products were purified (Qiа Quick PCR purification kit, Qiagen, Buenos Aires, Argentina) and sequenced using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Buenos Aires, Argentina) on an ABI PRISM 3130 Genetic Analyzer capillary DNA Sequencer (Applied Biosystems, Buenos Aires, Argentina). The primers used for sequencing were the same as those used for PCR. The nucleotide sequences obtained were compared with the NCBI entry of NR5A1 gene: NG_008176.1.

**In silico protein analysis**

The sequence homology-based tool SIFT (Sorting Intolerant from Tolerant; http://sift.jcvi.org/), version 2.0.6 and the structure-based tool PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/) were used to predict the pathogenicity of the missense variants p.Arg313His and p.Ser303Arg using default settings. The original sequence of the protein was obtained from the Ensembl and Uniprot/Swiss-Prot databases.

### Site-directed mutagenesis

For promoter activity experiments, an expression vector containing human wild type SF1 cDNA (p-SF1wt) was constructed in the pcDNA3 vector (Invitrogen, Buenos Aires, Argentina). The SF1 cDNA was PCR amplified using specific primers, SF1fw_pcDNA3 (5’TGGTGTGAGGGGTTTCTG3’) and SF1rev_pcDNA3 (5'GAGAGGAAGGGATGACC3’) carrying EcoRI and XhoI restriction enzyme sites, from RT-PCR of H295R cell line (human adrenocortical carcinoma cell line). The 1921-bp PCR product was 2% agarose gel-purified using the Zymoclean Gel DNA Recovery Kit (ZYMOR RESEARCH, Buenos Aires, Argentina). The fragment was digested with EcoRI/XhoI and cloned into the EcoRI and XhoI sites of the pcDNA3 vector.

The accuracy of the construct was confirmed by sequencing. NR5A1 expression vectors containing the p.Arg313His and p.Ser303Arg variants were generated by PCR-based site-directed mutagenesis (QuikChange Site-Directed Mutagenesis Kit, Stratagene, Buenos Aires, Argentina) with specific primers, using p-SF1wt as a template (p-R313H and p-S303R). The entire sequence of all mutant plasmids was confirmed by direct sequencing prior to functional studies.

### In vitro functional studies of NR5A1 mutations

All transient gene expression studies to assess NR5A1/SF1 function were performed in 24-well plates using Lipofectamine 2000 reagent (Invitrogen. Buenos Aires, Argentina) according to the manufacturer’s protocol. Each expression vector (p-SF1wt, p-R313H or p-S303R) was co-transfected into SMAT1 cell line (murine immature Sertoli cells) or into Y1 cell line (murine adrenocortical tumor cells) with reporter plasmid (PGL2, Promega, Buenos Aires, Argentina) containing hAMH promoter kindly provided by Dr. Rodolfo Rey (Centro de Investigaciones Endocrinológicas (CEDIE), Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina), p-PhAMH or with reporter plasmid (PGL3, Promega, Buenos Aires, Argentina) containing the h3BHSD2 promoter, p-PhBHSD2. Both promoters have response elements for SF1. The cells were lysed 48 hrs after transfection and assayed for luciferase activity (Dual Luciferase Reporter Assay System; Promega, Buenos Aires, Argentina). Co-transfection of CMV Renilla luciferase was used as a marker of transfection efficiency. Results are shown as the mean ± SEM of three independent experiments, each performed in triplicate and represents the ratio of luciferase activity.
of each SF1 and mutants compare to the empty vector. All data were standardized for Renilla activity. Statistical significance was examined using ANOVA test. Values of p<0.05 were considered significant.

In silico structural analysis of the wt protein and variants

Protein complexes in solution were prepared using as scaffold the X-ray crystallographic structure 1ZDT (chain B) of human SF1 wt LBD bound to a co-regulator peptide (NCoA-2, KENALLRYLLDKD) and a phospholipid ligand (di-palmityl-3-SN-phosphatidylethanolamine, PEF). Ser303Arg and Arg313His point mutations were introduced in silico with Discovery Studio Visualizer 3.5 [11]. Protonation states of the ionizable residues at pH 7.4 were defined by visual inspection after addition of H atoms missing in the 2.10 Å resolution crystal structure. Each structure was solvated by a truncated octahedral box of TIP3P water extended up to 12 Å around each complex, adding K+ ions to electroneutrality (7, 6, or 8 counterions respectively for wt, p.Ser303Arg and p.Arg313His). A standard classical minimization protocol (2500 steps relaxing solvent plus ions while maintaining the complex restrained with 500 kcal/mol Å, followed by 20000 steps of unrestrained optimization of the whole system) was applied under periodical boundary conditions with the amber module of the AMBER12 suite [12], assigning the ff03 force field to both SF1 and the co-regulator peptide, and the GAFF parameters to the phospholipid ligand. RESP atomic charges were also obtained with Gaussian09 rev. A.02 [13]. Long-range electrostatics was treated using the Particle-mesh Ewald (PME) approach [14], and a 10 Å cut-off was used for direct space interactions. Molecular dynamics simulations (MD) were run in explicit water for each complex at 300 K under the same conditions, using a 2 fs integration step and applying restrictions to H atoms with SHAKE algorithm [15]. After 100 ps equilibration in a NVT ensemble using a Langevin thermostat, 5 ns of NPT simulations were produced with pmemd from the AMBER12 suite of programs [12]. MD trajectory post-processing was conducted with the ptraj module of AmberTools 12 [12] and representative structures corresponding to the most populated cluster (among five clusters generated with the averaged-linkage algorithm) were obtained for each complex.

Results

Clinical features

The index case Family 1 [10], patient IV-2 was born with ambiguous genitalia (2 cm length phallus, labioscrotal folds, severe hypospadias, and small inguinal gonads). Hormonal determinations revealed elevated serum FSH and low AMH levels, and normal steroidogenic response to hCG stimulation (Table 1). Sex was re-assigned to male. Müllerian structures were present on ultrasound and confirmed by laparoscopy. A right gonad biopsy at 10 months of age revealed mild testicular dysgenesis, presence of Leydig cells, and few germ cells. His mother reported difficulties getting pregnant and presented low AMH and high basal FSH serum levels. His father referred normal sexual development and fertility. All affected individuals studied presented normal adrenal function.

Mutational analysis

The NR5A1 gene molecular study revealed the mutation c.938G>A (p.Arg313His) in heterozygous state in the first family and a novel mutation, c.909G>A (p.Ser303Arg) in heterozygous state in the second family. Both mutations are located in exon 5 in the highly conserved H5 of the LBD. In order to determine if these alterations are present with a high frequency in the general population (Single Nucleotide Polymorphism Database), we looked for these variations in the NCBI databases and ensembl genome browser and we did not find them, suggesting that these two variations would not be common polymorphisms. In addition, we search for the mutations in 100 healthy subjects (200 alleles) by DNA sequencing and no allele carrying this mutation was detected.

NR5A1 gene mutation prediction model

To assess the potential deleterious effect of the amino acid changes, we used two software programs, SIFT and PolyPhen-2. SIFT prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences. Arginine residue in position 313 and serine residue in position 303 are highly conserved between species. These mutations were evaluated to the option "affects protein function" with a highly deleterious tolerance index score of 0.00 and 0.02 respectively. The same assessment was performed with PolyPhen-2. This software predicts the possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations. Both mutations were predicted to be as "probably damaging" with a score of 1.000 and 0.999 respectively.

Structural analysis

The two variants containing point mutations p.Ser303Arg and p.Arg313His were compared to human SF1 wt. Both amino acid changes are located in the H5 helix of the LBD. In particular, p.Ser303Arg is located in the vicinity of the area of interaction with the co-regulator peptide (mainly delimited by H12, H3, and H4) and the p.Arg313His mutation is part of a salt bridge H2…H5 compacting the LBD structure of the receptor and stabilizing its active form [2,16].

From a comparison of the dynamical behavior of the protein backbone at the three SF1 complexes in solution emerges that whereas p.Ser303Arg displays a similar structural evolution than that of SF1 wt, for the p.Arg313His variant it takes longer (near 4 nanoseconds (ns)) to stabilize the 3D structure, which globally differs from the other two variants due to the loss of the salt bridge interaction (Figure 2A).

Since it has been reported that restrictions in the flexibility of NR5A1 receptors knock down their activation [17,18], we have compared this issue between the three variants using the root mean square fluctuations of the protein by residue (RMSF) calculated at the stabilized part of each 5 ns simulation (Figure 2B). As it can be immediately appreciated, whereas the p.Ser303Arg mutation turns the protein significantly more flexible throughout all its structure as compared to the wt (particularly in the H2-H3 loop and in the H12 helix), the p.Arg313His mutation turns, in contrast, the receptor more rigid in several regions.

The overall comparison of the tridimensional arrangement of alpha-helices and loops of the LBD across the three variants (Figures 2C and
2.830
3.671
27.59.

**Table 1:** Serum hormone levels in the affected individuals available for the study.

<table>
<thead>
<tr>
<th>Subject</th>
<th>CA (years)</th>
<th>LH(IU/l)</th>
<th>FSH(IU/l)</th>
<th>T(nmol/l)</th>
<th>E2 (pmol/l)</th>
<th>AMH (ug/dl)</th>
<th>Inhibit B (pg/ml)</th>
<th>Cortisol (pg/ml)</th>
<th>ACTH (pg/ml)</th>
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<tr>
<td>Family 1 (*)</td>
<td>II-1 46,XY</td>
<td>57</td>
<td>6.0</td>
<td>32.4</td>
<td>2.87</td>
<td>&lt;7.1</td>
<td>30</td>
<td>14.5</td>
<td>38.6</td>
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<tr>
<td></td>
<td>II-2 46,XY</td>
<td>31</td>
<td>2.3</td>
<td>4.6</td>
<td>2.97</td>
<td>&lt;2.9</td>
<td>60.8</td>
<td>12.1</td>
<td>25</td>
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<tr>
<td></td>
<td>III-3 46,XY</td>
<td>29</td>
<td>4.4</td>
<td>13.1</td>
<td>3.09</td>
<td>20</td>
<td>54.7</td>
<td>10.1</td>
<td>38.9</td>
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<tr>
<td></td>
<td>III-4 46,XX</td>
<td>27</td>
<td>12.1</td>
<td>47.5 (1.1-9.6)</td>
<td>0.56</td>
<td>&lt;9</td>
<td>&lt;0.3 (0-75)</td>
<td>8.2</td>
<td>22</td>
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<td></td>
<td>III-5 46,XX</td>
<td>25</td>
<td>11.7</td>
<td>25.1 (1.1-9.6)</td>
<td>0.31</td>
<td>28.3</td>
<td>5.9 (0-75)</td>
<td>5.6</td>
<td>15.5</td>
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<tr>
<td></td>
<td>IV-2 46,XY</td>
<td>0.03</td>
<td>9.6 (2.5 ± 1.7)</td>
<td>24.1</td>
<td>13.3 (2.4 ± 1.7)</td>
<td>24.5</td>
<td>1.04</td>
<td>3.95</td>
<td>&lt;9</td>
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<tr>
<td></td>
<td>IV-3 46,XX</td>
<td>3.8</td>
<td>&lt;0.1</td>
<td>12.4 (2.8±1.9)</td>
<td>&lt;0.05</td>
<td>&lt;2.9</td>
<td>360 (638-831)</td>
<td>31.9 (80-325)</td>
<td></td>
</tr>
<tr>
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<td>III-2 46,XY</td>
<td>25</td>
<td>12.3</td>
<td>17.8 (1.1-9.6)</td>
<td>ND</td>
<td>30.6</td>
<td>&lt;1.3 (0-75)</td>
<td>8.9</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>II-1 46,XY</td>
<td>0.3</td>
<td>3.75</td>
<td>13.6 (2.4±1.7)</td>
<td>0.72</td>
<td>1.36</td>
<td>133 (251-679)</td>
<td>39.9 (80-325)</td>
<td>11.7</td>
</tr>
</tbody>
</table>

Table 2: Structural analysis of the hydrogen bond interactions around each point mutation.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Residue:atom pairs</th>
<th>Distance (Å)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Ser303:O::Lys434:NH3</td>
<td>3.268</td>
<td>144.7</td>
</tr>
<tr>
<td></td>
<td>Ser303:OH1::Glu304:O</td>
<td>2.969</td>
<td>146.7</td>
</tr>
<tr>
<td></td>
<td>Ser303:C==Val307:NH</td>
<td>2.830</td>
<td>175.9</td>
</tr>
<tr>
<td>S303R</td>
<td>Arg303::Glu304:O</td>
<td>4.372</td>
<td>106.4</td>
</tr>
<tr>
<td></td>
<td>Arg303::Val307:NH</td>
<td>3.026</td>
<td>174.3</td>
</tr>
<tr>
<td>WT</td>
<td>Arg313:NE::Glu237:OE2</td>
<td>3.153</td>
<td>168.2</td>
</tr>
<tr>
<td></td>
<td>Arg313:NH2::Glu237:OE1</td>
<td>2.616</td>
<td>166.8</td>
</tr>
<tr>
<td></td>
<td>Arg313:NH2::Asp309:OD2</td>
<td>3.158</td>
<td>154.4</td>
</tr>
<tr>
<td></td>
<td>Arg313:NH1::Asp309:OD1</td>
<td>2.689</td>
<td>156.1</td>
</tr>
<tr>
<td></td>
<td>Arg313:NH2::Asp309:OD1</td>
<td>2.864</td>
<td>155.6</td>
</tr>
</tbody>
</table>

2D) highlights that both point mutations induce changes in the tertiary structure of the receptor around the ligand (H2-H3/H6-H7 loops, beta hairpin, and H6/H7 helices) and in the AF-2 domain (H4, H11, and H12), resulting in the latter in a different positioning of the co-activator peptide.

A closer inspection of the hydrogen-bond network surrounding each mutation promptly shows that whereas the interaction with Lys434 is the most affected for p.Ser303Arg (in comparison to SF1 wt), Arg313 ability of establishing hydrogen bonds with Glu237 and Asp309 in wt is completely lost when replaced by His313 in p.Arg313His (Table 2). Finally, a closer look in the comparison of the interactions with the ligand at the LBD across variants shows that as the p.Ser303Arg mutation affects SF1 interaction with the phospholipid mainly through changes in Lys440 positioning and the opening of the channel’s entrance defined by H2-H3 and H6-H7 loops (this resulting in PEF’s polar head more exposed to the solvent), p.Arg313His mutation does not produce significant changes on the ligand-receptor recognition (Figures 2E and 2F).

**Functional studies of NR5A1 mutation activities**

To examine the transcriptional activity of the SF1 mutants (p-R313H and p-S303R) and the wild type SF1 (p-SF1wt), we performed transient co-transfection assays with different SF1 target gene promoters (p-AMH and p-3BHSD2) in steroidogenic cell lines (SMAT1 and Y1).

In both steroidogenic cell lines, p-SF1wt significantly (p<0.05) increased reporter gene expression driven by the human AMH or 3BHSD2 promoters. Transfection with each of the two missense mutations p-R313H or p-S303R showed impaired transactivation activity compared to p-SF1wt (Figure 3).

In details, in the SMAT1 cell line the co-transfection with p-AMH and p-SF1wt significantly increased the luciferase activity (3.00 ± 0.99 AU, mean ± SEM, p <0.05 ANOVA) while mutants p-R313H and p-S303R significantly decreased luciferase activity compared to p-SF1wt (1.32 AU ± 0.05 and 1.09 AU ± 0.01 respectively, mean ± SEM, p<0.05 ANOVA) (Figure 3A). Similar results were obtained in the SMAT1 cell line with the co-transfection with p-3BHSD2; a significant increased luciferase activity in the presence of p-SF1wt (6.92 AU ± 0.25, mean ± SEM, p<0.05 ANOVA) while when assaying the effect of the mutants p-R313H and p-S303R a significant reduction of transactivation activity completely lost (Figure 3B).
capacity was observed compared to p-SF1wt (1.91 AU ± 0.07 and 2.00 AU ± 0.08 respectively, mean ± SEM, p<0.05 ANOVA) (Figure 3B).

In the Y1 cell line, similar responses as in the SMAT1 cell line were observed with p-Ph3BHSD2 and p-SF1wt (9.27 AU ± 0.54, mean ± SEM, p<0.05 ANOVA) and mutants p-R313H and p-S303R (4.55 AU ± 0.32 and 4.08 AU ± 0.17 respectively, mean ± SEM, p<0.05 vs. SF1wt, ANOVA) (Figure 3C). Transactivating studies of both mutants with the p-PhAMH in the Y1 cell line were not possible as the p-SF1wt did not increase luciferase activity.

Discussion

We are reporting the molecular characterization of two missense mutations (p-Arg313His and p.Ser303Arg) in the LBD of the SF1 gene, in two non-related 46,XY DSD patients, both in heterozygous state.

The clinical and biochemical phenotypes of these two 46,XY index patients were similar. Hormonal determinations showed normal basal and peak testosterone, high serum FSH and low serum AMH levels for the child’s age. Evidence of testicular dysgenesis and the presence of Müllerian
structures suggested a failure of embryonic fetal Sertoli cells to secrete AMH during the sensitive prenatal period, which is consistent with the low levels of AMH detected during infancy. The baby of the second family, evaluated at the age of minipuberty, also presented low serum inhibin B levels along with elevated FSH. As it was proposed in an aromatase deficient boy, inhibin B might be the major contributor in the regulation of serum FSH secretion in normal infant males [19].

Both patients were deficient of serum FSH secretion in normal infant males [19]. Both patients were initially assigned the female sex at birth, but after a careful evaluation, male sex re-assignation was recommended to, and accepted by the parents, on the basis of adequate basal T secretion for age, including normal response to hCG stimulation, and potential for successful intercourse in adulthood. This decision was also supported by the follow-up of other 46,XY DSD members of the first family who developed spontaneous male puberty, one with preserved fertility [10] and lack of reports of development of testicular tumors in 46,XY NR5A1 dysgenetic testes.

There was a striking variability among the affected relatives in the first family that range from severe ambiguous genitalia to normal male external genitalia and preserved fertility, as it was previously reported by us [10]. A wide phenotypic spectrum has been described in patients with heterozygous NR5A1 mutations including when the mutation is located in the LBD (Table 3) [5,6,9,20-32]. Pheno...
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Sex Assign.</th>
<th>Genital Phenotype</th>
<th>Puberty</th>
<th>Fertility</th>
<th>In silico prediction**</th>
<th>Functional characterization</th>
<th>Reference</th>
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<tr>
<td>p.Asp238Asn</td>
<td>Male</td>
<td>Normal Male</td>
<td>Spontaneous</td>
<td>Severe failure</td>
<td>Not done</td>
<td>Impaired transcription</td>
<td>[22]</td>
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<tr>
<td>p.Trp279Arg</td>
<td>Male</td>
<td>Micropenis, Perineal hypospadias</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Damaging</td>
<td>Not done</td>
<td>[23]</td>
</tr>
<tr>
<td>p.Arg281Pro</td>
<td>Male</td>
<td>Micropenis, Perineal hypospadias</td>
<td>Spontaneous</td>
<td>High sFSH</td>
<td>Asymptomatic carrier father</td>
<td>Damaging</td>
<td>Impaired transcription</td>
</tr>
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<td>p.Ser303Arg*</td>
<td>Male</td>
<td>Perineal hypospadias, EMS 8/12</td>
<td>Prepuberty</td>
<td>Prepuberty</td>
<td>Damaging</td>
<td>Impaired transcription</td>
<td>This report</td>
</tr>
<tr>
<td>p.Arg313Cys</td>
<td>Male</td>
<td>Glandular hypospadias</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not done</td>
<td>Impaired transcription</td>
<td>[23]</td>
</tr>
<tr>
<td>p.Arg313His</td>
<td>Male</td>
<td>Perineal hypospadias</td>
<td>Not reported</td>
<td>Asymptomatic carrier father</td>
<td>Not done</td>
<td>Not done</td>
<td>[26]</td>
</tr>
<tr>
<td>p.Arg313His*</td>
<td>Male</td>
<td>Perineal hypospadias</td>
<td>Spontaneous</td>
<td>Preserved</td>
<td>Damaging</td>
<td>Impaired transcription</td>
<td>[10] and this report</td>
</tr>
<tr>
<td>p.Gly328Val</td>
<td>Male</td>
<td>Perineal hypospadias</td>
<td>Spontaneous. Hypergon. hypogonadism</td>
<td>Azospermia</td>
<td>Not done</td>
<td>Impaired transcription</td>
<td>[27]</td>
</tr>
<tr>
<td>p.Val355Met*</td>
<td>Male</td>
<td>Micropenis, bilateral anoquia</td>
<td>Induced</td>
<td>Not reported</td>
<td>Not done</td>
<td>Impaired transcription</td>
<td>[28]</td>
</tr>
<tr>
<td>p.Cys370Trp</td>
<td>Male</td>
<td>DSD (EMS 3/12)</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Damaging</td>
<td>Not done</td>
<td>[29]</td>
</tr>
<tr>
<td>p.Leu376Phe</td>
<td>Female</td>
<td>DSD (female) Neonatal Citromegaly</td>
<td>Spontaneous (clitoromegaly)</td>
<td>Pubertal gonadectomy</td>
<td>Not done</td>
<td>Impaired transcription</td>
<td>[27]</td>
</tr>
<tr>
<td>p.Asp380Tyr</td>
<td>Female</td>
<td>DSD (female)</td>
<td>Not reported</td>
<td>Adolescent</td>
<td>Damaging</td>
<td>Not done</td>
<td>[30]</td>
</tr>
<tr>
<td>p.Leu437Gln</td>
<td>Male</td>
<td>Perineal hypospadias</td>
<td>Induced</td>
<td>Not reported</td>
<td>Not done</td>
<td>Impaired transcription</td>
<td>[31]</td>
</tr>
</tbody>
</table>

Table 3: Missense mutations in the SF-1 ligand binding domain of 46,XY patients
*In silico structural analysis performed;
**Analyzed using SIFT and/or PolyPhen-2 tools.
sFSH: serum FSH.
EMS: External Masculinization Score (minimum: 1, maximum: 12) [32]

gene (p.Arg313His) previously described without any functional study performed, and a novel missense mutation (p.Ser303Arg), both in heterozygous state, located in the LBD. *In vitro* and *in silico* experiments argued for their functional impact and also provided insight into the structure-function relationship of the SF1 protein. Finally the present study reinforces the concept of the wide variability in the clinical phenotype in affected 46,XY DSD patients.

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References


