Spontaneous virilization around puberty in NR5A1-related 46,XY sex reversal: additional case and a literature review

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Abstract. A heterozygous NR5A1 mutation is one of the most frequent causes of 46,XY DSD (disorders of sex development). We here reported a NR5A1-related 46,XY DSD patient, who first received endocrinological attention at 10 years of age for clitoromegaly. The patient had been reared as a girl, and no signs of virilization had been detected before. On examination, her clitoris was 35 mm long and 10 mm wide, with Tanner 3° pubic hair. Urogenital sinus and labial fusion was absent, while her uterus was found to be severely hypoplastic. Her basal testosterone level was 94.8 ng/dL, suggesting the presence of functioning Leydig cells. Gonadal histology revealed bilateral dysplastic testes consisting of mostly Sertoli cell-only tubules and Leydig cell hyperplasia. Novel heterozygous Arg313Leu substitution in NR5A1 was identified in the patient. Literature search confirmed twelve other cases of this scenario, namely, severe under-virilization in utero followed by spontaneous virilization around puberty in NR5A1-related 46,XY DSD. Of interest, Leydig cell hyperplasia was documented in 6 out of 9 patients for whom testicular histology was available. To keep in mind about the possible restoration of Leydig cell function around puberty, even in patients without discernible in utero androgen effect, may be of clinical significance, because it will give a great impact on the judgement about sex assignment.

Key words: Clitoromegaly, Disorders of sex development, Dysplastic testis, Leydig cell hyperplasia, 46,XY sex reversal 3
with a birth weight of 3,054 g and had no other significant medical history. At referral for endocrinological evaluation, she had a height of 159 cm (+2.5 SD) and a low-pitched voice, without acne. Breast development of Tanner 2° was noticed. Her clitoris was 35 mm long and 10 mm wide, with Tanner 3° pubic hair. External urethral meatus and vaginal introitus were separated, while labial fusion was absent. Bone age was 12.0 years for female when assessed by Tanner-Whitehouse II RUS scoring system standardized for Japanese population. Imaging studies revealed a severely hypoplastic uterus (Fig. 1A). Serum gonadotropin levels were elevated: LH 16.7 IU/L and FSH 75.4 IU/L. Basal testosterone was 94.8 ng/dL, which further increased to 203 ng/dL following human chorionic gonadotropin stimulation. Peripheral blood chromosome analysis with 30 metaphases showed 46, XY karyotype.

On laparotomy at 11 years of age, dysplastic testes were identified bilaterally and then resected. Left testis, measured as 45 × 30 × 17 mm, was located at the inguinal region whereas right testis, measured as 27 × 18 × 15 mm, was found in the retroperitoneal space. Neither of the testes looked like ovary, and any ovarian tissue was not found in the abdomen. Histological examination (Fig. 2) showed the dysplastic nature of the testes, namely, tubules consisted of mostly Sertoli cells with a thickened basement membrane. In addition, moderate Leydig cell hyperplasia (LCH) was observed in both testes.

She is now 27 years old, 176.5 cm tall, and has been on estrogen supplementation for 12 years. Her uterus is still hypoplastic, although significant growth is observed following the longstanding estrogen therapy (Fig. 1B). Introduction of Kaufmann therapy has been reserved, because she experienced lower abdominal pain when a cycle of gestagen was administered. Until now, she has never experienced menstruation. She has never developed adrenal failure, even at the time of influenza-like illness. Although scrutiny with provocation tests has not been carried out, basal ACTH and cortisol levels in the late morning have been normal with ACTH at 11.1–17.1 pg/mL and cortisol at 7.0–7.5 μg/dL.

Molecular analysis revealed a novel heterozygous c.938G>T (p.Arg313Leu) substitution in the NR5A1 gene. This novel mutation replaced a basic amino acid (Arg) with a neutral one (Leu) and was not found in 220 normal control alleles. Arg313 resides in the ligand-binding domain and is highly conserved in different species (Fig. 3). Other missense mutations at this position, namely p.Arg313His and p.Arg313Cys, have been repeatedly reported, suggesting Arg313 as a mutational hotspot [16-19]. In silico analysis using PolyPhen-2 resulted in probably damaging (score 1.000). Her parents were not subjected to the analysis.

An informed consent form to permit publication was obtained from the patient.
Discussion

Our patient presented with a rapid virilization beginning at 10 years of age, without any discernible in utero androgen effects on her genitalia, such as urogenital sinus and labial fusion. Meanwhile, the regression of Müllerian derivatives was substantially achieved, as shown by a severely hypoplastic uterus (Fig. 1A). These indicate that, while Sertoli cell function was rather preserved, Leydig cell function was profoundly impaired during her fetal period. Nevertheless, her Leydig cells later began to produce sufficient amounts of testosterone to cause apparent virilization.

An identical scenario, namely, spontaneous virilization around puberty in NR5A1-related 46,XY sex reversal, has been anecdotally described [3-12]. We collected 12 other cases from the literatures (Table 1). All of these cases had female-type external genitalia at birth and were raised as females, although subtle in utero androgen effects were present in some patients. In most patients, as in our patient, virilization around puberty triggered a diagnosis of NR5A1 mutation [4-12].

It is conceivable that restoration of Leydig cell function around puberty may have occurred more frequently. In cases who presented with subtle virilization at birth, it is probable that a NR5A1 mutation was revealed early, and gonadectomy had been carried out before puberty. Once gonadectomized, the fate of Leydig cell function thereafter remains unknown. In turn, the possibility of spontaneous virilization around puberty must have a great impact on the decision of sex assignment. If Leydig cell function restores frequently, incomplete in utero androgen effect should not be easily connected to female sex assignment. Accordingly, the long-term natural course of Leydig cell function in NR5A1-related 46,XY sex reversal should be clarified.

It is unclear why spontaneous virilization occurs around the age of puberty. At present, the most plausible explanation is the shift of Leydig cells from the fetal to adult type [8]. Fetal and adult Leydig cells represent different cell populations [20, 21] and the latter is thought to be less dependent on NR5A1 products during steroidogenesis, partly because the liver receptor homolog 1 (NR5A2) takes over deficient NR5A1 in adult Leydig cells [22]. In addition, a recent study in male mice demonstrated that NR5A1 product has distinct primary func-

Fig. 3  A) c.938G>T substitution in the patient’s NR5A1 gene is shown with sequence chromatogram. This substitution is located in the 5th exon.
B) The p.Arg313Leu mutation is located in the ligand-binding domain.
C) The mutated arginine residue at position 313 is highly conserved among various species. Data were obtained from Vertebrate Multiz Alignment & Conservation in UCSC Genome Browser.
### Table 1  
**Hitherto reported cases of NR5A1-related 46,XY sex reversal who showed spontaneous virilization around puberty**

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Urogenital sinus</th>
<th>Labial fusion</th>
<th>Testosterone (ng/dL)</th>
<th>LH/FSH (IU/L)</th>
<th>AMH* (pmol/L)</th>
<th>Müllerian structures</th>
<th>Gonadal histology</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hasegawa [3] [2004]</td>
<td>27 y</td>
<td>absent</td>
<td>absent</td>
<td>57</td>
<td>10/36</td>
<td>NA</td>
<td>absent</td>
<td>severely hyalinized tubules, few Sertoli cells, few Leydig cells</td>
</tr>
<tr>
<td>Lourenço [4] [2009]</td>
<td>18 y</td>
<td>present</td>
<td>present</td>
<td>42</td>
<td>18.0/36.3</td>
<td>NA</td>
<td>NA</td>
<td>germ cell aplasia, Leydig cell hyperplasia</td>
</tr>
<tr>
<td></td>
<td>12 y</td>
<td>NA</td>
<td>NA</td>
<td>185</td>
<td>21.7/96.9</td>
<td>NA</td>
<td>NA</td>
<td>disorganized tubules, Leydig cell hyperplasia</td>
</tr>
<tr>
<td>Tajima [5] [2009]</td>
<td>12 y</td>
<td>absent</td>
<td>absent</td>
<td>78</td>
<td>14.4/59.8</td>
<td>NA</td>
<td>absent</td>
<td>rare germ cells, loose interstitium with a few cluster of Leydig cells</td>
</tr>
<tr>
<td>Warman [6] [2011]</td>
<td>12.2 y</td>
<td>present</td>
<td>NA</td>
<td>260</td>
<td>4.8/30.2</td>
<td>16.7</td>
<td>absent</td>
<td>tubules with wide lumen, incomplete spermatogenesis, Leydig cell hyperplasia</td>
</tr>
<tr>
<td>Barbaro [7] [2011]</td>
<td>13 y</td>
<td>absent</td>
<td>absent</td>
<td>410</td>
<td>5.3/42</td>
<td>0.71</td>
<td>absent</td>
<td>normal germ cells number in left testis, scarce germ cell in right testis, pronounced Leydig cell hyperplasia</td>
</tr>
<tr>
<td>Cool [8] [2012]</td>
<td>14 y</td>
<td>NA</td>
<td>NA</td>
<td>210</td>
<td>2.3/35.9</td>
<td>NA</td>
<td>absent</td>
<td>no spermatogenesis</td>
</tr>
<tr>
<td>Tantawy [9] [2012]</td>
<td>10 y</td>
<td>absent</td>
<td>absent</td>
<td>181.5</td>
<td>8.4/44.5</td>
<td>&lt;0.1</td>
<td>absent</td>
<td>Sertoli cell-only tubules, no germ cells, very scarce Leydig cells</td>
</tr>
<tr>
<td>Siklar [10] [2014]</td>
<td>8.8 y</td>
<td>NA</td>
<td>NA</td>
<td>10</td>
<td>1.5/7.5</td>
<td>NA</td>
<td>absent</td>
<td>NA</td>
</tr>
<tr>
<td>Woo [11] [2015]</td>
<td>12.3 y</td>
<td>absent</td>
<td>NA</td>
<td>414</td>
<td>13/77</td>
<td>NA</td>
<td>absent</td>
<td>NA</td>
</tr>
<tr>
<td>Domenice [12] [2016]</td>
<td>13 y</td>
<td>present</td>
<td>NA</td>
<td>16.7 y</td>
<td>415</td>
<td>24/69</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>16.7 y</td>
<td>NA</td>
<td>NA</td>
<td>155</td>
<td>24/69</td>
<td>NA</td>
<td>NA</td>
<td>Sertoli cell-only tubules, moderate Leydig cell hyperplasia</td>
</tr>
<tr>
<td>This case [2018]</td>
<td>10 y</td>
<td>absent</td>
<td>absent</td>
<td>94.8</td>
<td>16.7/75.4</td>
<td>NA</td>
<td>hypoplastic uterus</td>
<td>Sertoli cell-only tubules, moderate Leydig cell hyperplasia</td>
</tr>
</tbody>
</table>

NA: not available, *AMH: anti-Müllerian hormone. Reference interval of AMH for 9–18 year G3 male is 30–423 pmol/L (ref. 27).
tions on fetal and adult Leydig cells: it regulates differentiation of the former, whereas it regulates progenitor cell formation and/or survival in the latter [23].

As shown in Table 1, LCH or ‘cluster of Leydig cells’ was documented in 6 out of 9 cases whose histological findings were provided [4-8]. Although no strict LCH definition has been established, this term seems to comprise two conditions, namely, LCH with nodular growth to form testicular mass, and LCH with diffuse sheets of Leydig cells occupying the interstitium [24, 25]. Apparently, LCH observed in our patient, and others with NR5A1 mutation, corresponds to the latter category of LCH. The association of this type of LCH with androgen insensitivity syndrome and unclassified DSD has been reported [25]. It is tempting to think LCH observed repeatedly in the patients with NR5A1 mutations may be closely related to restoration of Leydig cell function.

Distinct from LCH, Leydig cells with cytoplasmic lipid droplets, which is reminiscent of STAR mutation, have been proposed to be intrinsic to NR5A1 mutation [2, 26]. However, this finding was absent in the patients listed in Table 1, including our patient. In addition, Leydig cells with lipid droplets have only been observed in younger patients [2, 26]. Therefore, lipid accumulation in Leydig cells does not seem to be relevant to virilization around puberty. Albeit highly speculative, Leydig cells with lipid droplets may occur mainly in fetal Leydig cells, not in adult cells.

In Table 1, all patients who showed spontaneous virilization had elevated FSH levels. In addition, antimüllerian hormone levels evaluated in three patients were lower than reported reference interval [27]. These data suggest the impaired Sertoli cell function. In the reviewed data of seventy-two 46,XY DSD patients with NR5A1 mutations, müllerian derivatives are present in only 24% of cases, whereas FSH levels were persistently elevated after puberty [12]. Taken together, whereas Sertoli cell function seems to be preserved during fetal period, it will be severely damaged after puberty in 46,XY DSD patients with NR5A1 mutations.

The presence of an ovary in our patient is an obscure finding. If the hernia content at 3 years of age was truly an ovary, then she would have both testis and ovary. The presence of ovotestis was reported in a 46,XY patient with NR5A1 deletion [28]. In addition, in several 46,XX patients with particular NR5A1 mutations, such as p.Arg92Trp and p.Arg92Gln, variable degree of testicular formation has been reported [13-15]. Testicular formation in 46,XX individuals is supposed to derive from the disruption of ovarian specific developmental pathway, the Wnt/β-catenin pathway, which will normally suppress the expression of testicular genes [13, 14]. Therefore, a coexistence of testis and ovary in our patient may be anticipated, especially under the situation of tissue mosaicism.

**Conclusion**

Spontaneous and progressive virilization around puberty may occur in NR5A1-related 46,XY sex reversal, sometimes with LCH. Thus, verification of NR5A1 mutation in 46,XY DSD patients has a substantial clinical impact on decision making during sex assignment.

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**Declaration of Conflict of Interest**

The authors declared that they have no competing interests.

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