Clinical Features and Diagnostic Approach in Patients with Undervirilised External Male Genitalia

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Abstract

Background. Sex development in humans is a complex and multiple step process. SRY gene on Y chromosome has met the criteria for a testis determining factor, however subsequent stages of differentiation require the expression of several other genes. Male pseudohermaphroditism refers to genetically XY male with differentiated testes and varying degree of undermasculinization of the external genitalia.

Aim. Our study aimed at exploring the clinical characteristics, cytogenetic and SRY gene analyses in a group of 14 pediatric patients with male pseudohermaphroditism.

Material and Methods. Clinical presentations were: micropenis and/or penoscrotal/perineal hypospadia in 86%, altered appearance of the scrotum in 79% and Mullerian duct remnants in 50% of the patients.

Results. Cytogenetic analyses revealed a normal male karyotype in all patients. SRY gene was found positive in all 14 patients.

Conclusion. We concluded that SRY gene analysis in patients with male pseudohermaphroditism confirms the clinical diagnosis and directs further investigations. SRY gene analysis is a quick and relatively simple diagnostic method that can be included at the early stage diagnostic of sex differentiation disorders.

Introduction

Sex development in humans is a complex and multiple step process. Two major phases have been recognized: sex determination, the initial event that determines development of gonads into testis or ovaries and sex differentiation, the subsequent events that produce either male or female sexual phenotype (1). A single gene on the Y chromosome termed SRY gene (Sex Determining Region on Y chromosome) was shown to trigger testicular determination (2, 3) acting as a transcription regulator through local effects on chromatin structure (4). However, subsequent stages of differentiation require the expression of several other genes such as the AMH gene (that encodes the Anti-Mullerian hormone) (5-7) and genes that control the production and action of androgens (8-10). Disorders of sex differentiation can be divided into two major categories: 1. Normal complement of sex chromosomes, but other genetic/constitutional disorders leading to abnormal sex differentiation and 2. Abnormal complement of sex chromosomes (11). The “undervirilised male”, classically termed the “male pseudohermaphrodite” (MPH), is a genetically XY male with differentiated testes and varying degree of undermasculinization of the external genitalia (11). This condition accounts for up to 60% of all genital ambiguities (12,13). Testosterone biosynthetic de-
fects, Leidig cell unresponsiveness to gonadotropin stimulation, and secondary or tertiary deficiencies of the hypothalamic-pituitary-gonadal axis are infrequently recognized as causes of undervirilisation (14-18) whereas some forms of androgen resistance (5 alpha-reductase deficiency, partial androgen insensitivity syndrome) are commonly recognized causes of insufficient virilisation in a XY male (13,14,19,20). Our research focuses on the clinical features of XY male patients with undervirilised external genitalia, their diagnostic workup and analysis of the SRY gene.

Methods

Study was performed on 14 children with ambivalent external genitalia, patients of the Departments of endocrinology and genetics and neonatology at the University Pediatric Clinic in Skopje, Republic of Macedonia. Mean age of diagnosis was 12.8 ± 21.6 months. Diagnosis was set according to the appearance of the external genitalia, cytogenetic analysis, hormonal status and ascertainment of the gonadal sex.

Clinical examination

A comprehensive clinical examination was performed in all patients. The genital ambiguity inclusive of phallic length, palpable gonads, position of the urethral opening or presence of urogenital sinus, and hypertrophic labia or labioscrotal folds were recorded to describe the stage of virilisation (21).

Hormonal analyses

17 hydroxyprogesterone (17OHP) was tested in all patients using ELISA method.

Imaging techniques

Internal genital structures were analyzed by pelvic and abdominal ultrasonography and voiding cystourethrogram. Gonadal sex was ascertained using ultrasonography and/or gonadal biopsy.

Cytogenetic analyses

Peripheral blood lymphocytes were examined by G banding (banding resolution of 400 bands, average number of observed metaphase preparations, 50).

DNA extraction

Genomic DNA was extracted from 2-6 mL ethylenediamine tetraacetate-containing blood using a standard phenol-chloroform procedure from peripheral blood leucocytes (22, 23).

Polymerase chain reaction amplification

Two sets of oligonucleotide primers were used: XES7/XES2 and SRY 1F/SRY 2R. XES7 5'-GAC AAT GCA ATC ATA TGC TTC TGC-3'/XES2 5'-CTG TAG CGG TCC CGT TGT GCG GTG-3' amplify a 609 bp fragment that spans almost the entire open reading frame (ORF) of the SRY gene (24-27). SRY 1F 5'-CAG TGT GAA ACG GGA GAA AAC AGT-3'/SRY 2R 5'-CTT CCG ACG AGG TCG ATA CTT ATA-3' amplify a 270 bp fragment (518-788 bp) that mainly encompasses the HMG-box domain, an evolutionary highly conserved motif that codes for a protein with DNA-binding characteristics (26, 27). The primers were synthesized in Sigma Genosys (Sigma-Aldrich Corporation, St.Louis, Missouri, USA). Amplification of a 165 bp fragment of the angiotensinogen gene was used as a control. The sequences of the primer pairs used to amplify the internal controls were: F 5'-TCA CAT ATG GTA TGA CCC TC-3'/R 5'-TTG TAC CAG CTC ACT ACC TA-3'.

The PCR amplification was performed in a final volume of 50 μL, the reaction mixture consisting of 300-500 ng genomic DNA, 50 pmol of each specific primer and 30 pmol of the control primers, 1.5 U AmpliTaQ Gold polymerase (Applied BioSystems, Foster City, California, USA), 2 mM MgCl₂, 200 mM 4 x dNTP, commercial PCR buffer. The amplification was carried out with a DNA thermal cycler (Perkin Elmer 480 version 2; Perkin Elmer Corporation, Waltham, Massachusetts, USA); amplification conditions were previously reported by our study group (27).

Analysis of the Polymerase Chain Reaction Amplified Products

All PCR products (10μL) were electrophoresed on a 2% agarose gel in 1 x TBE buffer stained by ethidium bromide and visualized under UV light. Several precautions were taken to avoid false-positive results (27).

Results

The clinical data, karyotype and SRY gene analyses of the 14 patients with male pseudohermaphroditism are summarized in Table 1.

There was wide variation in the age of presen-
Table 1: Clinical data, karyotype and SRY gene in 14 patients with male pseudohermaphroditism.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at diagnosis (months)</th>
<th>External genitalia</th>
<th>Internal genital structures</th>
<th>Palpable gonads (y/n)</th>
<th>Karyotype</th>
<th>SRY gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.0</td>
<td>MP, HS, LS</td>
<td>male urethra, short vagina</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2.</td>
<td>0.33</td>
<td>MP, HL</td>
<td>male urethra, vagina</td>
<td>y, right only</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>3.</td>
<td>10.0</td>
<td>MP, BS, HS</td>
<td>male urethra</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>4.</td>
<td>48.0</td>
<td>MP, BS</td>
<td>no VCUG</td>
<td>y, left only</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>5.</td>
<td>7.0</td>
<td>MP, HS</td>
<td>male urethra</td>
<td></td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>6.</td>
<td>12.0</td>
<td>LS</td>
<td>shorter male urethra</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>7.</td>
<td>0.53</td>
<td>BS</td>
<td>female urethra, SUG</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>8.</td>
<td>0.6</td>
<td>BS, MP</td>
<td>female urethra, SUG, vagina, suspected uterus</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>9.</td>
<td>74.4</td>
<td>HS, MP</td>
<td>male urethra, SUG, vagina</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>10.</td>
<td>12.0</td>
<td>LS, MP, HS</td>
<td>shorter male urethra, small vagina</td>
<td>y, right only</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>11.</td>
<td>5.0</td>
<td>HPS, HS</td>
<td>male urethra, hypoplastic vagina, suspected uterus</td>
<td>y, right only</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>12.</td>
<td>0.53</td>
<td>LS, MP</td>
<td>female urethra</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>13.</td>
<td>8.0</td>
<td>LS, MP</td>
<td>shorter male urethra, small vagina</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
<tr>
<td>14.</td>
<td>0.47</td>
<td>HS</td>
<td>female urethra</td>
<td>y</td>
<td>46 XY (+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Legend: MP-micropenis; HS-hypospadia; LS-labiform scrotum; BS-bifid scrotum; HPS-hypoplastic scrotum; VCUG-voiding cystourethrogram; SUG-sinus urogenitalis.

The extent of masculinization in these patients varied considerably from slightly decreased (example cases No. 3 and 5) to a complete intersexuality of the external genitalia (example cases No. 7 and 8). Gonads were verified as testes by palpatory finding and/or by ultrasonography bilaterally or uni-

evaluation of these 14 patients from 10 days to 6.2 years with a mean of 12.8 ± 21.6 SD months. All patients except case No.10 were reared as males. 17OHP values were in reference range in all patients, thereby the diagnosis of congenital adrenal hyperplasia (CAH) was excluded. Initial presentations were: micropenis and/or penoscrotal/perineal hypospadia in 12/14 (86%) and altered appearance of the scrotum (labiform, bifid or hypoplastic) in 11/14 (79%) of the patients. The extent of masculinization in these patients varied considerably from slightly decreased (example cases No. 3 and 5) to a complete intersexuality of the external genitalia (example cases No. 7 and 8). Gonads were verified as testes by palpatory finding and/or by ultrasonography bilaterally or uni-
Male pseudohermaphroditism (MPH) is the most common group of sex differentiation disorders and accounts for more than 50% cases of intersex disorders (12, 13). Idiopathic MPH is a heterogeneous condition, even within families with a history of this condition (10, 28). Our group consisted of 14 patients with variable degree of sexual ambiguity. Micropenis and/or penoscrotal/perineal hypospadia as initial clinical manifestations were detected in 86%, a percentage higher than described in other studies (28). Mullerian duct derivatives were detected by a genitogram in 50% of patients, a percentage similar to other studies (28). The extent of masculinization in these patients varied considerably from slightly decreased to a complete intersexuality of the external genitalia. Gonads were detected unilaterally or bilaterally in all patients, the percentage of bilateral detection corresponds with previous reports (8). The SRY gene was detected in all patients that confirmed the clinical diagnosis—male pseudohermaphroditism. We did not find a SRY negative patient in this group; therefore we concluded that the absence of this gene in MPH is rare. However, although rare, mutations in the promoter region of the gene cannot be excluded (29). It is evident that further genetic study of genes downstream of SRY is necessary in these patients such as WT1, SF1, SOX9, DAX1, AMH, AMHR (5, 7, 30-37). Due to the large number of reported cases of androgen insensitivity syndrome (AIS) and 5 alpha reductase type 2 deficiency in series of patients with MPH (8-10, 19, 20) molecular analysis of androgen receptor gene (AR) and SRD5A2 gene mutations should be considered. The assignment of sex for rearing in cases of male pseudohermaphroditism must be guided by the etiology of the genital malformation, the anatomic condition, the potential for reconstructive surgery, the pubertal response of the external genitalia to endogenous and exogenous testosterone, as well as family’s considerations (9). Management of ambiguous genitalia in the newborn requires a multidisciplinary approach through the diagnostic procedure, the choice of sex assignment, and the treatment strategy (9, 38). Diagnostic workup should comprise: physical examination, verification of the genital and gonadal status by ultrasonography and genitogram, cytogenetic, hormonal and molecular analyses (9, 13, 14, 21). SRY gene analysis in the group of MPH should be a starting point analysis and basis for further diagnostic workup. Identification of the SRY gene and positive testosterone test in this group of patients can lead to a recommendation for corrective surgical intervention and male gender assignment. In cases of female sex of rearing, a 5 alpha reductase type 2 deficiency should be excluded to avoid unpleasant clitoromegaly and pubic hair growth in puberty and gonadal extirpation should be performed prior to puberty (9, 13). Gender assignment should be made as soon as possible after birth, but absolutely should be made by 18 months of age, when children develop gender identity; therefore appropriate early diagnosis is essential (39-41).

Our study is a clinically oriented and brings in correlation variable clinical manifestations of MPH with genetic diagnosis. Although a heterogeneous group, a significant percentage of MPH (around 50%) (13, 14) is caused by different forms of insensitivity to androgen action (8-10, 19, 20). Our study was not aimed to explore androgen insensitivity in our patients. We are aware that, if more sensitive genetic diagnostic tools are applied, a more precise etiological differentiation in our group of patients would be
possible. However, a thorough clinical evaluation, successful therapeutic trial with testosterone combined with adequate surgical corrective procedure could lead to an external genital appearance and function compatible with family's satisfaction.

As a conclusion we can say that diagnostic procedure for gender assignment is an immediate obligation of the clinician. It involves many clinical, biochemical, imaging and molecular diagnostic procedures. SRY gene analysis in patients with male pseudohermaphroditism confirms the clinical diagnosis and directs further investigations. SRY gene analysis is a quick and relatively simple diagnostic method that can be included at the early stage diagnostic procedure of sex differentiation disorders.

References


