Tall Stature and Poor Breast Development after Estrogen Replacement in a Hypergonadotrophic Hypogonadic Patient with a 45,X/46,X,der(X) Karyotype with SHOX Gene Overdosage

ABSTRACT

SHOX is exclusively expressed in the developing distal limb bones of human embryos and in the first and second pharyngeal arches. It works as a promoter for linear growth and as a repressor of growth plate fusion. It was reported, recently, that SHOX overdosage and gonadal estrogen deficiency have led to tall stature due to continued growth. We report, in the present study, a female patient with 45,X/46,X, psu idic(X)(pter→q21::q21→pter) karyotype, tall stature, and hypergonadotrophic hypogonadism without Turner stigmas. She did not present breast development even after long term therapy with high estrogen doses. Fluorescence in situ hybridization depicted the presence of three copies of SHOX gene. Microsatellite studies showed paternal origin of der(X). Further studies in similarly affected patients will clarify if the absence of breast development, despite previous high-dose estrogen treatment, is associated to triple copy of SHOX gene. (Arq Bras Endocrinol Metab 2008; 52/8:1282-1287)

Keywords: SHOX overdosage; Tall stature; Gonadal dysgenesis

RESUMO

Estatura Alta e Hipodesenvolvimento Mamário após Reposição Estrogênica em Paciente com Hipogonadismo Hipergonadotrófico e Cariótipo 45,X/46,X, der(X) com Superdosagem do Gene SHOX.

O gene SHOX é expresso, exclusivamente, no primeiro e no segundo arcos faríngeos, assim como nas extremidades dos ossos dos membros em embriões humanos. SHOX normalmente atua como um promotor para o crescimento linear e como um repressor do fechamento da placa de crescimento. Recentemente, foi descrito que o excesso da proteína SHOX associada à deficiência estrogênica gonadal leva à estatura alta devido ao contínuo crescimento. Neste estudo descrevemos uma paciente do sexo feminino com cariótipo 45,X/46,X,psu idic(X)(pter→q21::q21→pter), estatura alta, hipogonadismo hipergonadotrófico e sem estigmas de Turner. A paciente não apresentou desenvolvimento de mamas, mesmo depois do tratamento prolongado com altas doses de estrógenos. FISH evidenciou a presença de três cópias do SHOX. Estudo de microsatélites demonstrou o origem paterna do der(X). Estudos futuros em pacientes com semelhanças clínicas esclarecerão se a ausência de desenvolvimento de mamas, apesar do tratamento com altas doses de estrógenos, está associada à tripla cópia do SHOX. (Arq Bras Endocrinol Metab 2008; 52/8:1282-1287)

Descritores: Overdosagem de SHOX; Estatura alta; Deficiência estrogênica gonadal
INTRODUCTION

At the end of short X and Y chromosome arms there is a common portion named short arm pseudautosomal region (PAR-1) spanning 2.6 Mb. PAR-1 escapes from X inactivation process and recombines during male meiosis (1). Individuals with absence of this region present short stature which indicates that this region is related to growth (2,3). In 1997, two different groups described, simultaneously, a gene responsible for stature, SHOX (“Short Stature Homeobox-Containing Gene”) (2,3), located on the PAR-1. SHOX is composed by 7 exons with 40 kb in length (4), that encodes two different proteins, one with 292 and other with 225 amino acids due to an alternative splicing (2,3). It is exclusively expressed in the first and second pharyngeal arches and in the developing distal limb bones of human embryos (5). SHOX normally functions as a promoter for linear growth and as a repressor for growth plate fusion and skeletal maturation (6). SHOX haploinsufficiency due to deletions or mutations leads to short stature and skeletal abnormalities such as in Turner syndrome, or Madelung deformity of the forearm, such as in Léri-Weill dyschondrosteosis.

Recently, patients with karyotype alterations involving sexual chromosomes resulting in SHOX overdose were described in the literature (7-10). This fact associated to estrogen deficiency owing to gonadal dysgenesis seems to be the cause of excessive growth, especially in the distal limb bones, in the middle to late teenage females (7,10,11).

In the present study, we report a 45,X/46,X,der(X) female patient with tall stature, low body mass index (BMI), no breast development or Turner’s stigmata and gonadal insufficiency in whom a SHOX gene over-dosage was suspected.

METHODS

Cytogenetic Analysis

Chromosome metaphase spreads prepared from peripheral blood lymphocyte cultures of the patient and her parents were analyzed by conventional staining, G and C banding techniques. The kit Vysis® Kallman Region Probe (Vysis Inc., Abbott Laboratories, Illinois, USA) and the biotinylated LLNOYCO3’M’34F5 cosmid containing exons III to VIb of SHOX gene were used for the FISH methodology. The analysis was performed using the Karyotyping Software Macktype v.5.4.1 and Mackprobe v. 4.0 (Perceptive Scientific Instruments Inc., UK) for FISH.

Molecular Analysis

Genomic DNA was obtained from peripheral blood leukocytes using the Salting-Out technique (12). The microsatellite study was carried out using the panel for the X chromosome presented in the kit Linkage Mapping set v.2.5 MD10 (PE Applied Biosystems, The Perkin-Elmer Corporation, CA, USA). The PCR products were submitted to electrophoresis in the ABI PRISM 310 automatic sequencer (PE Applied Biosystems, The Perkin-Elmer Corporation, CA, USA) and the analysis was made by GeneScan software (PE Applied Biosystems, The Perkin-Elmer Corporation, CA, USA).

CASE REPORT

The present study was approved by the Ethics Committee of the Hospital das Clínicas, The University of Sao Paulo Medical School. Written consent was obtained from the patient and their parents.

A 16 year-old Brazilian girl was referred due to absence of secondary sexual development. She was born at 40 weeks of gestation after an uncomplicated pregnancy, a single daughter of non consanguineous parents. At birth, she weighted 3.250 g and measured 52 cm. Her growth chart showed that she grew at the 50th percentile until 9 months of age, at the 75th percentile from 9-33 months, and after 3 years of age at > 95th percentile. She underwent spontaneous pubarche at 12 years, without telarche. She presented menarche at 14 years, after estrogen replacement without adequate breast development. At 16 her height was 183 cm, 20 cm above her target height, with an eunuchoid habitus and her BMI was 15 Kg/m². Puberty evaluation disclosed Tanner II breast development, Tanner V pubic hair and normal female external genitalia (Table 1). Basal serum hormone data revealed elevated gonadotropins (LH= 40 U/L and FSH= 85 U/L), low estrogen levels, normal PRL, GH, IGF1 and IGFBP3 levels.

RESULTS

Cytogenetic analysis showed a 45,X[4]/46,X,der(X) [46] karyotype (Figure 1). The parents’ karyotypes study was normal. FISH analysis revealed an extra copy...
### Table 1. Clinical data before and after hormonal treatment.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>SD (height)</th>
<th>Weight (kg)</th>
<th>Breast (Tanner)</th>
<th>Pubic hair (Tanner)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.8</td>
<td>174.8</td>
<td>+ 3.5</td>
<td>43</td>
<td>I</td>
<td>IV</td>
<td>Conjugated estrogen 0.625 mg</td>
</tr>
<tr>
<td>16.5</td>
<td>183</td>
<td>+ 3.5</td>
<td>50</td>
<td>II</td>
<td>V</td>
<td>Valerate of estradiol 2 mg Levonorgestrel 0.25 mg</td>
</tr>
<tr>
<td>16.8</td>
<td>183.5</td>
<td>+ 3.5</td>
<td>51</td>
<td>II</td>
<td>V</td>
<td>Valerate of estradiol 2 mg Levonorgestrel 0.25 mg</td>
</tr>
<tr>
<td>17</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>II</td>
<td>NA</td>
<td>Ethnyl estradiol 70 ug Ciproterone acetate 4 mg</td>
</tr>
<tr>
<td>17.5</td>
<td>184</td>
<td>+ 3.6</td>
<td>51</td>
<td>III</td>
<td>V</td>
<td>Ethnyl estradiol 70 ug Ciproterone acetate 4 mg</td>
</tr>
<tr>
<td>18</td>
<td>184.2</td>
<td>+ 3.7</td>
<td>52</td>
<td>III</td>
<td>V</td>
<td>Ethnyl estradiol 35 ug Ciproterone acetate 2 mg</td>
</tr>
<tr>
<td>18.6</td>
<td>184.4</td>
<td>+ 3.7</td>
<td>52</td>
<td>III</td>
<td>V</td>
<td>Valerate of estradiol 2 mg Levonorgestrel 0.25 mg</td>
</tr>
</tbody>
</table>

NA = Not available

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**Figure 1.** GTW-banded Karyotype: 46,X,psuidic(X)(pter→ q21::q21→pter).
of \textit{SHOX} and \textit{KAL} genes and X centromere (Figures 2 and 3) in the der(X), showing that the der(X) is a psu idic(X)(pter→q21::q21→pter). Microsatellite study revealed the paternal origin of psu idic(X)(pter→q21::q21→pter) (Figure 4). The markers DXS991 and DXS986 localize on Xp11.21 and Xq21.1, respectively, were not informative.

**DISCUSSION**

Recently, it has been reported that \textit{SHOX} over dosage and gonadal estrogen deficiency leads to tall adult height (7,9). These findings suggest that \textit{SHOX} over dosage in combination with gonadal dysgenesis leads to continuous growth since early infancy until late adolescence, since \textit{SHOX} functions as a repressor for growth plate fusion, in contrast with skeletal maturing effect of estrogens (10).

Here we reported a patient with a 45,X[4]/46,X,psu idic(X)(pter→q21::q21→pter)[46] karyotype and go-
nadal dysgenesis, tall stature, no Turner stigmas and poor breast development after estrogen replacement. The altered X chromosome has a paternal origin, which contains a partial Xq deletion, two centromeres and a Xp duplication resulting in an extra SHOX gene.

To date, six female patients with an extra copy of SHOX gene in a der(X) were described (Table 2). Three of them had tall stature as well as the present case. However, three patients present normal height despite the extra copy of SHOX associated to estrogen deficiency indicating that an extra copy of SHOX is not sufficient for the development of tall stature (7-10). On the other hand, Kanaka-Gantenbein, 2004 suggested that SHOX triplication per se may induce the tall stature, since their patient presented tall stature in the absence of estrogen deficiency (15). These controversial reports may be due to the fact that the mechanism of SHOX action still remains unclear.

Our patient showed poor breast development after estrogen treatment. Normally, these patients presented adequate breast development after estrogen treatment (9,10,13). However, Nakamura et al. also reported a patient with gonadal dysgenesis and an extra copy of SHOX who had poor breast development after estrogen therapy (8). The reason for the poor breast development in these two patients is still unexplained. Further studies in similarly affected patients will clarify if the absence of breast development despite high-dose estrogen treatment is related to the extra copy of SHOX gene.

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Table 2. Phenotype of SHOX dosage in female patients.

<table>
<thead>
<tr>
<th>Age (Yrs)</th>
<th>Height (cm SD)</th>
<th>HH</th>
<th>Turner Stigmas</th>
<th>Breast Development (after estrogen)</th>
<th>Cytogenetic Data</th>
<th>Der(X) Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>172 + 2.9</td>
<td>Present</td>
<td>Mild webbed neck</td>
<td>Full development</td>
<td>45,X(40)/46,X,der(X)(pter→q13 or q21.2→pter)</td>
<td>Paternal</td>
<td>(10)</td>
</tr>
<tr>
<td>20</td>
<td>174 + 2</td>
<td>Present</td>
<td>Absent</td>
<td>Tanner II</td>
<td>46,X,der(X)(pter→q21.2→pter)</td>
<td>NR</td>
<td>(8)</td>
</tr>
<tr>
<td>20</td>
<td>161.9 + 0.8</td>
<td>Present</td>
<td>Absent</td>
<td>Sufficient development</td>
<td>45,X(28)/46,X,psu idic(X)(q28)</td>
<td>NR</td>
<td>(9)</td>
</tr>
<tr>
<td>20</td>
<td>166 + 1.5</td>
<td>Present</td>
<td>Absent</td>
<td>Normal</td>
<td>46,X,rec(X)dup(Xp)inv(X)(p11.22q21.2)/46,XX</td>
<td>Maternal</td>
<td>(13)</td>
</tr>
<tr>
<td>14</td>
<td>172 + 2</td>
<td>Present</td>
<td>Multiple naevi, high arched palate</td>
<td>NR</td>
<td>46,X,der(X)(pter→q21.2→pter)</td>
<td>NR</td>
<td>(7)</td>
</tr>
<tr>
<td>20</td>
<td>NR - 1.2</td>
<td>Present</td>
<td>Absent</td>
<td>NR</td>
<td>45,X(6)/46,X,der(X)(pter→q21.1→q22.3→pter)</td>
<td>NR</td>
<td>(14)</td>
</tr>
<tr>
<td>16</td>
<td>183 + 3.5</td>
<td>Present</td>
<td>Absent</td>
<td>Tanner II</td>
<td>45,X(4)/46,X,psu idic(X)(pter→q21→pter)</td>
<td>Paternal</td>
<td>Present Study</td>
</tr>
</tbody>
</table>

HH: hypergonadotropic hypogonadism; NR: not reported.

REFERENCES


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