Follow up of precocious pseudopuberty associated with isolated ovarian follicular cysts

K A Rodriguez-Macias, E Thibaud, M Houang, C Duflos, C Beldjord, R Rappaport

Abstract
The clinical outcomes of seven girls presenting with pseudosexual precocity caused by isolated autonomous ovarian follicular cysts are presented. Six of the seven girls, aged 11 months to 6.9 years, had a unilateral ovarian cyst detected by ultrasound at the first acute episode. Plasma oestradiol was raised in only five of the cases, but all had a low response to luteinising hormone releasing hormone stimulation. Follow up lasted for up to eight years with recurrent episodes of variable frequency and severity in all seven patients. Evidence of McCune-Albright syndrome appeared later in only three patients. It could not be predicted from the initial symptoms or the clinical course. Mutations of the Gα protein leading to activation were investigated in the lymphocytes and ovarian and bone tissues of four patients. Only one patient showed a mutation in bone tissue. Close follow up with repeated searches for skeletal lesions remains necessary since the distribution of somatic mutations cannot be assessed by molecular studies. Most patients with recurrent ovarian cysts require a conservative approach.

Keywords: pseudopuberty; ovarian follicular cyst; G protein mutation

Table 1 Acute ovarian follicular cyst before age of puberty: clinical presentation at diagnosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Chronological age (years)</th>
<th>Bone age (years)</th>
<th>Vaginal bleeding</th>
<th>Pubertal Tanner stage</th>
<th>Growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.9</td>
<td>5.9</td>
<td>+</td>
<td>B3 P2</td>
<td>Increased</td>
</tr>
<tr>
<td>2*</td>
<td>1.6</td>
<td>2.0</td>
<td>+</td>
<td>B2 P1</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>5.9</td>
<td>6.0</td>
<td>+</td>
<td>B3 P1</td>
<td>Increased</td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>2.0</td>
<td>0</td>
<td>B2 P2</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>5.4</td>
<td>5.3</td>
<td>+</td>
<td>B2 P1</td>
<td>Increased</td>
</tr>
<tr>
<td>6*</td>
<td>0.9</td>
<td>1.3</td>
<td>+</td>
<td>B2 P2</td>
<td>Increased</td>
</tr>
<tr>
<td>7*</td>
<td>2.6</td>
<td>2.6</td>
<td>+</td>
<td>B2 P3</td>
<td>Increased</td>
</tr>
</tbody>
</table>

*Clinical evidence of McCune-Albright syndrome during follow up.
of 100 μl containing 67 mM Tris HCl (pH 8.8), 16.6 mM SO₄(NH₄), 2.5 mM MgCl₂, 10 ml dimethyl sulfoxide, 1 mM each of all four deoxynucleotides, 0.2 mM each of the primers, 2.5 units Taq polymerase (Perkin-Elmer Cetus, USA) and 200 ng template. Samples were heated at 94°C for 5 min and at 57°C for 1 min in the first round of denaturation-annealing, followed by 40 cycles of 30 seconds at 94°C, 30 seconds at 57°C, and 30 seconds at 72°C, and a final elongation for 7 min at 72°C in a Perkin-Elmer 9600 thermal cycler.

The Gs mutations at codons 201 and 227 were screened by denaturating gradient gel electrophoresis (DGGE) analysis using chemical clamps instead of guanine–cytosine tails (15). Computer programs kindly provided by Lerman and colleagues were used to predict the melting behaviour of the fragments and to determine the appropriate denaturant concentration range. An aliquot (1/10) of the amplified product was clamped by ultraviolet irradiation (365 nm) and loaded on a 6% polyacrylamide gel with a linearly parallel gradient of 30–60% denaturant. The fragment spanning exon 9 was separated by electrophoresis for 7 hours at 160 V in a gradient containing 50–80% denaturant.

The amplified PCR products were purified on a microcon-100 column (Amicon, USA) and sequenced. For this, 10 ng purified DNA was mixed with 5 pmol primer in the ABI PRISM dRhodamine Dye terminator cycle sequencing kit (Perkin Elmer/Applied Biosystems, USA). The cycle sequencing conditions were 93°C for 3 min, followed by 25 cycles of 94°C for 30 seconds, 55°C for 5 seconds, and 60°C for 4 min. The fluorescent products were analysed on an ABI 377 machine.

**Results**

**INITIAL PRESENTATION**

Six of the seven girls presented with early vaginal bleeding. Breast development to stages B2 or B3 was recent in all of them and lasted for only a few weeks. Four girls had pubic hair (P2 or P3). The growth rate during the previous year was above normal in five patients, but their bone ages were all normal (table 1). None of the patients showed any of the pigmented skin lesions or bone dysplasia characteristic of McCune-Albright syndrome.

Plasma oestradiol concentration was raised (30–270 pg/ml) in five patients, but was within the normal prepubertal range (<15 pg/ml) in two (patients 5 and 6). There were no plasma FSH and LH responses to LHRH stimulation in any of the girls, including those with prepubertal plasma oestradiol concentrations.

Pelvic ultrasound examination revealed unilateral ovarian cysts of 25–65 mm in six patients. Patient 6 was found to have a 15 mm diameter cyst only at the second episode of vaginal bleeding six months later (table 2). The contralaterals were 9–22 mm (upper limit for normal prepubertal ovary 25 mm), suggesting a lack of gonadotropin stimulation. All patients had a stimulated uterus over 35 mm long.

FOLLOW UP

Follow up is shown in fig 1. The clinical signs caused by oestrogen activity and the ultrasound evidence of ovarian cyst regressed within 2–4 weeks, except in patient 1. The ovarian cyst in this patient persisted and increased in size to 90 mm. Her symptoms were unusually severe as her ovarian activity remained sustained with raised plasma oestradiol concentrations for seven months. Therefore a unilateral oophorectomy was performed. This was followed by regression of the clinical oestrogen activity and normalisation of the response to LHRH. Further follow up was not possible. Patient 2 was followed for 18 months, during which time she had five more episodes of oestrogenic activity. Patients 3 and 5 were followed for three and six years, respectively, with only one recurrent episode, and have now entered normal puberty. Patient 4 had no recurrence during a five year follow up. Patients 6 and 7 were followed for eight years and had multiple episodes of ovarian activation. McCune-Albright syndrome was diagnosed in patients 2, 6, and 7 at 18, 15, and 40 months, respectively, after the initial vaginal bleeding. This diagnosis was based on the development of bone dysplasia detected by repeated skeletal radiographs, as there were no characteristic skin lesions. These patients had numerous (4–11) recurrent episodes of bleeding which were more frequent than in patients 3, 4, and 5, who had long, uneventful follow up periods. They also were among the youngest patients.
increased plasma oestradiol concentrations during the early phase of central precocious puberty, together with low plasma LH and FSH basal and peak response to LHRH stimulation. However, the plasma oestradiol measurement may be misleading, since two girls had prepubertal plasma oestradiol values in spite of clinical evidence of increased oestrogen activity. These results contrasted with the complete suppression of gonadotropin response to LHRH stimulation. The apparent discrepancy between these results may be caused by variations in the plasma oestradiol concentrations, as suggested by the persistence of oestrogen stimulated cells in the vaginal smear. This type of finding should therefore be taken as strong evidence against central precocious puberty. It shows the need for an early LHRH stimulation test in patients whose clinical profile suggests autonomous ovarian activation.

Ovarian ultrasonography performed at the time of the initial episode is critical for diagnosis, as the presence of an ovarian cyst \( \geq 9 \) mm in diameter is a strong indicator of pseudosexual precocity with autonomous ovarian activation. A study of 32 girls under eight years old found that 23 had true isosexual precocity, with 69% having small cysts less than 9 mm in diameter, and one with a larger cyst (15 mm). In contrast, the four patients with pseudosexual precocity had unilateral ovarian cysts with diameters of 15–64 mm. The lack of contralateral ovarian enlargement was also considered to indicate pseudosexual precocity with autonomous ovarian activation.

Our patients were initially referred to us because of recent evidence of acute sexual precocity. Although surgery was necessary because of ovarian torsion in one case, conservative follow up should be possible for most of these patients, providing ultrasound monitoring is included.

Three patients showed evidence of McCune-Albright syndrome. As described, they showed sexual precocity before the development of bone lesions and skin café au lait pigmentation. Although multiple episodes of vaginal bleeding and breast stimulation occurred before McCune-Albright syndrome was diagnosed, the frequency, number, and characteristics of such episodes did not appear to be specific or predictive of the outcome. However, the symptoms of two patients (2 and 6) were most severe. Therefore, a process self limited to the ovary could not be ascertained in any of these patients during their initial follow up. The LH response to LHRH stimulation remained suppressed several months after the initial episode, although the clinical evidence of oestrogen activity had disappeared in the three patients who developed McCune-Albright syndrome. Whether this is a characteristic feature of more severe and extensive cases remains to be evaluated in larger series of patients. Diagnosis of McCune-Albright syndrome requires a prolonged clinical follow up with repeated skeletal radiographs.

The molecular basis of McCune-Albright syndrome is a mutation in the \( \alpha \) subunit of the...
arginine 201 to cysteine mutation of the Gs protein, which couples cell surface receptors to the stimulation of adenyl cyclase, Gs. These mutations lead to the constitutive activation of adenyl cyclase in various tissues, including the ovary. A previous study showed that specific regions of abnormal ovarian tissue contained a higher proportion of mutant cells, by hybridisation of DNA with a mutant oligonucleotide probe. These results indicated a correlation of the abundance of mutant alleles with the pathological abnormalities in ovarian tissue. However, no such mutations were found in blood cells, dysplastic bone, or the café au lait spots of several patients. There were no mutations in the blood cells of our three affected patients, even in the patient with documented McCune-Albright syndrome.

Laboratory tests therefore have limitations, perhaps because of technical difficulties or the great variations in distribution and density of Gα mutations within and between tissues. Hence only positive results should be considered as informative for clinical purposes. An arginine 201 to cysteine mutation of the Gs protein was found in bone tissue, but not in any other tissues. This mutation causes a notable decrease in intrinsic GTPase activity, prolonging the lifetime of the active conformation. The protein was found in bone tissue, but not in any other tissues. This mutation causes a notable decrease in intrinsic GTPase activity, prolonging the lifetime of the active conformation.

We conclude that premature, intermittent ovarian activity mimicking precocious puberty may be caused by autonomous ovarian activation. The initial clinical and ultrasound picture is characteristic. A lack of plasma LH and FSH responses to LHRH, when the plasma oestrogen is normal prepubertal, is useful for diagnosing autonomous ovarian function. This episode may be a single event or there may be recurrences at unpredictable intervals, perhaps accompanied by other manifestations of the McCune-Albright syndrome. Sustained oestrogen activity rarely requires testolactone treatment. Ovarian surgery should be avoided, or should be very conservative in case of ovarian torsion. This is most important for follow up and treatment. Prepubertal girls are likely to present over several years of follow up with a continuous spectrum of clinical symptoms, from an isolated ovarian functional cyst to the full blown picture of McCune-Albright syndrome.

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