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Endocrine and molecular investigations in a cohort of 25 adolescent males with prominent/persistent pubertal gynecomastia

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SUMMARY

Pubertal gynecomastia is a common condition observed in up to 65% of adolescent males. It is usually idiopathic and tends to regress within 1–2 years. In this descriptive cross-sectional study, we investigated 25 adolescent males with prominent (>B3) and/or persistent (>2 years) pubertal gynecomastia (P/PPG) to determine whether a hormonal/genetic defect might underline this condition. Endocrine investigation revealed the absence of hormonal disturbance for 18 boys (72%). Three patients presented Klinefelter syndrome and three a partial androgen insensitivity syndrome (PAIS) as a result of p.Ala646Asp and p.Ala45Gly mutations of the androgen receptor gene. The last patient showed a 17α-hydroxylase/17,20-lyase deficiency as a result of a compound heterozygous mutation of the CYP17A1 gene leading to p.Pro35Thr(P35T) and p.Arg239Stop(R239X) in the P450c17 protein. Enzymatic activity was analyzed: the mutant protein bearing the premature stop codon R239X showed a complete loss of 17α-hydroxylase and 17,20-lyase activity. The mutant P35T seemed to retain 15–20% of 17α-hydroxylase and about 8–10% of 17,20-lyase activity. This work demonstrates that P/PPG had an endocrine/genetic cause in 28% of our cases. PAIS may be expressed only by isolated gynecomastia as well as by 17α-hydroxylase/17,20-lyase deficiency. Isolated P/PPG is not always a 'physiological' condition and should thus be investigated through adequate endocrine and genetic investigations, even though larger studies are needed to better determine the real prevalence of genetic defects in such patients.

INTRODUCTION

Pubertal gynecomastia is a common condition observed in up to 65% of adolescent males. It results from a proliferation in the glandular component of the breast, usually because a transient relative imbalance between androgens and estrogens. Pubertal gynecomastia is generally idiopathic and the breast development is most often Tanner stage B2 (Tanner, 1962). As it tends to regress within 1–2 years, pubertal gynecomastia is considered as a benign condition and is regarded as a part of normal development in pubertal males (Moore *et al.*, 1984).

If male breast development is over B3-B4, however, or if it persists beyond 2–3 years, it may be the sign of an endocrine disorder, becoming a source of anxiety, self-consciousness, embarrassment and severe psychological discomfort. In these cases, determining the pathogenesis of pubertal gynecomastia becomes mandatory.

In prominent and/or persistent pubertal gynecomastia (P/PPG), the imbalance between the actions of androgens and estrogens on male breast tissue may be attributable to an excess of estrogen production, a decrease in androgen production or action, or drugs. Estrogen-producing gonadal or adrenal tumors and endocrine disorders thus have to be looked for, and patients should be carefully questioned about drug use. Moreover, aromatase excess has recently been reported as a cause of P/PPG (Fukami *et al.*, 2011). Careful attention should be paid to the patient's morphotype and the testes should be examined to exclude Klinefelter syndrome. An underlying disorder of sex differentiation (DSD) such as partial androgen insensitivity syndrome (PAIS) or 17-β-hydroxysteroid dehydrogenase defect should also be ruled out (Narula & Carlson, 2007). In addition, environmental factors (Ma & Geffner, 2008) may be implicated. Any of these conditions can cause excessive estrogen and/or deficient androgen activity.

The aim of our study was to identify the causes of prominent/persistent pubertal gynecomastia (P/PPG) in adolescent males, particularly hormonal/genetic defects.

SUBJECTS AND METHODS

Patients

This descriptive cross-sectional study included all adolescent males referred to the Pediatric Endocrine Clinic of the Montpellier University Hospital between January 2011 and January 2014 for prominent and/or persistent pubertal gynecomastia. The gynecomastia was evaluated by inspection and palpation as described by (Braunstein, 2007) to distinguish between pseudogynecomastia and true gynecomastia. The selection criteria included the following: (i) Tanner pubertal status ≥2 (Tanner, 1962), (ii) lack of associated genital abnormalities, (iii) prominent breast development (>B3), or (iv) persistence beyond 2 years. All patients were asked about the use of drugs known to be involved in the development of gynecomastia, i.e., spironolactone, cimetidine, ketoconazole, and antiandrogens (Deepinder & Braunstein, 2012), as well as the abuse of alcohol, marijuana, heroin, amphetamines, and anabolic steroids (Goldman, 2010). Renal and liver diseases were excluded for all patients.

Approval for this study was first obtained from the appropriate institutional review board and all patients gave signed informed consent.

Anthropometric data

Standing height was measured to the nearest 0.1 cm with a stadiometer (Seca, Semur-en-Auxois, France). Weight was measured on a weight scale with a precision of 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Grades of obesity were defined on the basis of the cut-off points used by the Obesity Task Force and derived from Cole's work (Cole *et al.*, 2000). Pubertal development was assessed by breast stages 1–5 of the Tanner classification (Tanner, 1962).

Radiological investigation

Bone age (BA) was determined using the Greulich and Pyle method and testicular ultrasonography was performed for all patients to exclude a testicular tumor.

Hormonal (biological) studies

The following hormones were measured at baseline for all patients: estradiol (E2) (bioMerieux, Craponne, France), testosterone (T), Δ4-androstenedione (Δ4) (Immunotech, Marseille, France), dehydroepiandrostenedione (DHEA) (Beckman Coulter, Villepinte France), luteinizing hormone (LH), follicle-stimulating hormone (FSH) (bioMerieux, Craponne, France), prolactin (BRAHMS, Asnières-sur-Seine, France), and thyroid-stimulating hormone (TSH) (Roche, Boulogne-Billancourt, France).

The ACTH test was performed in one patient with the measurement of cortisol (Roche), 17-OHP (CIS bio international, Gif-sur-Yvette, France), progesterone (Roche), and DHEA before and after stimulation.

Alpha-fetoprotein and human chorionic gonadotropin (hCG) were evaluated for all patients (Roche).

The respective intra-assay and inter-assay coefficients of variation (CVs) were as follows: E2: 4.2 and 5.1%, T: 7 and <4%, 17-OHP: 2.7 and 2.9%, DHEA: 6.8 and 7.9%, Δ4: 5.6 and 6%, progesterone: 2.5 and 3.5%, LH: 4.8 and 6.6%, FSH: 4.9 and 3.5, PRL: 2.1 and 2.9%, TSH: 3 and 6%, and cortisol: 2.6 and 3.8%. The detection limits were as follows: E2: 5 pg/mL, T: 0.04 ng/mL, 17-OHP: 0.1 ng/mL, DHEA: 0.3 ng/mL, Δ4: 0.04 ng/mL, progesterone: 0.09 nmol/L, LH: 0.1 U/L, FSH: 0.1 U/L, prolactin: 0.24 ng/mL, TSH: 0.005 μU/mL, and cortisol: 0.04 μg/dL.

Karyotyping

Karyotyping was performed when the clinical and biological investigations suggested a genetic disorder; i.e., when the basal FSH level was very high and/or the testicular volume was small. It was also performed for a patient in whom we suspected a steroidogenesis defect.

Molecular analysis

Androgen receptor (AR) gene

After polymerase chain reaction (PCR) amplification of exons 1–8 of the AR using the Taq PCR Master Mix kit from Qiagen (Courtaboeuf, France), we performed direct sequencing using the BigDye terminator v1.1 kit (Applied Biosystems, Foster City, CA, USA) and an ABI Prism310 Genetic Analyzer (Applera, Courtaboeuf, France), as reported elsewhere (Philibert *et al.*, 2010). In cases of mutation, PCR and sequencing of the DNA sample were repeated twice to confirm the finding and rule out any PCR-generated errors. Every PCR product was sequenced with forward and reverse primers. The amino acid numbering for the AR was based on the NCBI reference sequence NM_000044.2 and the AR database (Gottlieb *et al.*, 2012).

CYP17A1 gene

The CYP17A1 gene was studied when a P450c17 enzymatic deficiency was suspected on the basis of the patient's hormone profile. After obtaining informed consent, genomic DNA was extracted from peripheral leukocytes and then used to perform PCR exonic amplification of the gene, as previously described (Biason-Lauber *et al.*, 1997). The PCR products were sequenced using the Big Dye Terminator Cycle Sequencing Kit and analyzed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Reference Sequence: RefSeq NM_000102.3. Primer sequences are available upon request.

Concerning the molecular analysis, patient sequences were compared with the sequence of a control patient with normal XY DNA.

Expression studies

Expression studies were performed as previously described (Rosa *et al.*, 2010). Briefly, wild-type CYP17A1 cDNA (originally from Michael R. Waterman) was inserted into a pcDNA3.1 vector after addition of an N-terminal myc-tag. Mutant cDNAs were constructed using the QuikChange II site-directed mutagenesis kit from Stratagene (La Jolla, CA, USA). Introduction of the mutations was confirmed by sequencing. Wild-type or mutant cDNA was transiently transfected into confluent COS-1 cells using TransFast transfection reagent (32 μl transfection reagent/3.6 μg DNA). To standardize the steroid production, cells were cotransfected 1:2 with β-galactosidase (β-gal). Forty-eight hours after transfection, steroidogenic precursors (progesterone for 17α-hydroxylase activity and 17-OHP for 17,20-lyase activity)

were added at concentrations of 1.0 μM, which is a concentration that is close to the Km of this enzyme. Six hours after addition of the precursor, supernatants were removed and kept frozen at −20 °C until measured. β-gal activity was measured using the β-galenzyme assay system (Promega Corp., Madison, WI, USA). The secreted steroids, 17-OHP (reflecting 17α-hydroxylase activity) and DHEA (reflecting 17,20-lyase activity), were measured in duplicate by radioimmunoassay, using commercial kits from Diagnostic System Laboratories (Morwell Diagnostics, Zurich, Switzerland). Values were standardized for β-galactivity and expressed as a percentage of wild-type activity.

To further explore the disease mechanism, we analyzed the RNA stability of the R239X mutant carrying the premature stop codon to check for nonsense-mediated decay (NMD). To inhibit translation, 3×10^7 COS1 cells were pelleted, washed once with phosphate-buffered saline, and resuspended in medium containing 28 μg of cycloheximide/ml. To subsequently remove the cycloheximide, the cells were pelleted, washed twice with phosphate-buffered saline, and resuspended in medium without the inhibitor (Rajavel & Neufeld, 2001). The stabilization of RNA was checked by end-point reverse transcriptase-polymerase chain reaction (RT-PCR) after RNA extraction, using the RNeasy kit (Qiagen, Hilden, Germany). Primers and amplification conditions are available upon request. GAPDH was used as internal control.

RESULTS

Over the 3-year study period, 148 boys were referred to our center for pubertal gynecomastia. Among them, 25 had P/PPG and were analyzed. The clinical characteristics and BA of these 25 patients are presented in Table 1. The patients' mean age (SD) was 14.5 ± 1.1 years at the time of diagnosis (range 12.8–16.5 years) with a mean bone age (SD) of 14.4 ± 1.2 years. Breast development was scored B3 in 22 patients (88%), all of whom presented a persistent form of gynecomastia. Only three patients (patients 23, 24, and 25) had a score of B4. The pubertal status was scored at Tanner stages 2, 3, 4, and 5, respectively, in two (8%), nine (36%), 11 (44%), and three (12%) patients.

Obesity was noted for 11 of the boys (44%); seven were grade 1 (28%) and four were grade 2 (16%). The mean BMI (SD) was 23.2 ± 5.2 kg/m^2.

The hormonal characteristics of these 25 patients with prominent and/or persistent pubertal gynecomastia are presented in Table 2. The endocrine investigations showed normal FSH and LH secretion in all patients, except patients 19 and 20, who presented high basal gonadotropin levels. The testosterone level was correlated with the Tanner stage in all patients, with a mean value of 2.87 ± 1.74 ng/mL. Estradiol (mean value: 20.29 ± 11.73 pg/mL), prolactin (mean value: 7.85 ± 3.72 ng/mL) and TSH (mean value: 1.92 ± 0.69 mUI/L) were in the normal range and thus excluded estrogen-secreting tumor, prolactinoma, and thyroid disorders. Alpha-fetoprotein (mean value: 1.43 ± 1.01 ng/mL) and hCG (data not shown) levels were normal in all patients, excluding endocrine tumor.

The very high basal gonadotropin levels in patients 19 and 20, and the small testis found for these two patients and for patient 21, raised the hypothesis of chromosomal disorders. The karyotype was 47,XXY for these three patients, which led to the diagnosis of Klinefelter syndrome.

Our attention was drawn by the family history of patients 22, 23, and 24. Two of these patients (23, 24) had the distinction of being twins and, in addition, their mother complained about very sparse pubic hair from early puberty. The third patient (22) had a maternal uncle who presented gynecomastia and never had children. A p.Ala646Asp mutation in the androgen receptor gene was found in patients 23 and 24, and this mutation was

Table 1 Clinical characteristics and bone age of the 25 patients with prominent/persistent pubertal gynecomastia (P/PPG). Pubertal status is based on genital staging

Patient	CA (years)	BA (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Percentiles	Obesity grade	Pubertal status (Tanner stage)	Breast development
1	14.3	14	175.0	80.0	26.1	>97th	1	4	B3
2	12.8	13	162.0	41.0	15.6	10th	0	3	B3
3	13.5	13	158.5	56.8	22.6	95th	0	2	B3
4	12.8	13	160.5	57.8	22.4	>97th	1	3	B3
5	14.5	17	183.0	109.0	32.5	>97th	2	5	B3
6	14.2	15	174.0	68.0	22.5	95th	0	4	B3
7	13.0	14	169.5	71.0	24.7	>97th	1	4	B3
8	14.6	16	172.5	57.4	19.3	50th	0	5	B3
9	15.8	15	162.8	101.6	38.3	>97th	2	4	B3
10	15.3	14	166.0	52.5	19.0	30th	0	3	B3
11	13.8	13	165.9	63.4	23.0	>97th	1	4	B3
12	14.6	15	176.0	78.0	25.2	>97th	1	4	B3
13	14.8	14	170.0	56.0	19.4	50th	0	3	B3
14	13.9	14	166.0	47.0	17.0	25th	0	4	B3
15	15.3	15	162.3	40.3	15.3	<3th	0	5	B3
16	12.9	13	172.5	81.7	27.5	>97th	2	2	B3
17	15.4	14	165.5	73.7	26.9	>97th	1	3	B3
18	14.8	17	168.2	59.0	20.8	75th	0	3	B3
19	13.7	13	170.0	66.4	23.0	97th	0	3	B3
20	14.1	15	176.0	59.5	19.2	50th	0	3	B3
21	13.6	15	169.0	81.6	29	>97th	2	3	B3
22	15.2	15	178.0	69.5	21.9	75th	0	4	B3
23	16.5	15	176.0	82.6	26.2	>97th	1	4	B4
24	16.5	15	170.5	68.5	23.7	90th	0	4	B4
25	15.4	14	173.5	60.0	20.0	60th	0	4	B4

CA, chronological age; BA, bone age; BMI, body mass index. Patients with genetic defects have been highlighted.

Table 2 Hormone characteristics of the 25 patients with P/PPG for testosterone (T), estradiol (E2), follicle-stimulating hormone (FSH), luteininzing hormone (LH), prolactin (PRL) and thyroid-stimulating hormone (TSH)

Patient	T (ng/mL) (*N* = 3.5–11)	E2 (pg/mL) (*N* = 20–60)	T/E2 (*N* = 175–183)	FSH (UI/L) (*N* = 1.5–12)	LH(UI/l) (*N* = 1.5–6)	PRL(ng/mL) (*N* < 20)	TSH (mUI/L) (*N* = 0.1–3.5)	Genetic defect
1	3.50	19.1	183	3.0	1.3	7.9	2.09	
2	2.62	18.2	144	4.3	3.0	8.6	3.05	
3	1.06	9.0	118	1.5	1.5	10.2	1.11	
4	2.83	9.0	314	0.4	1.8	3.4	2.04	
5	4.63	9.2	503	1.4	6.8	7.1	2.18	
6	4.69	36.5	128	1.4	2.1	8.7	2.89	
7	3.68	20.3	181	2.9	2.2	6.9	1.20	
8	8.41	39.0	216	1.3	2.5	5.7	1.80	
9	4.35	8.0	544	3.9	2.2	4.2	2.69	
10	2.43	13.5	180	3.3	0.8	5.0	2.02	
11	2.95	9.5	310	2.3	0.9	5.4	1.37	
12	3.84	10.5	366	1.1	0.6	5.1	0.78	
13	2.51	9.0	279	1.6	1.9	3.2	1.39	
14	4.54	18.5	245	2.2	2.4	6.4	1.62	
15	6.20	14.5	428	3.5	2.8	5	3.11	
16	1.05	31.9	33	3.1	1.4	4.1	1.75	
17	1.63	9.0	181	0.9	1.1	5.7	2.79	
18	2.56	12.0	213	3.2	1.9	9.4	1.64	
19	1.79	10.0	179	79.9	25.6	13.6	2.65	Klinefelter
20	1.90	36.5	52	43.7	9.3	6.1	1.08	Klinefelter
21	1.46	15	97	10.3	5.1	9	1.20	Klinefelter
22	3.61	20	180	3.8	2.7	12	2.60	AR
23	4.05	16.4	247	1.2	3.4	16	2.30	AR
24	3.76	22	171	2.2	5	16.4	1.10	AR
25	1.60	47.8	33.4	7.5	12	7.7	1.67	CYP17A1

AR, androgen receptor gene mutation, CYP17A1, CYP17A1 gene mutation. N: normal references correspond to normal post-pubertal values. Patients with genetic defects have been highlighted.

previously reported in a patient with infertility (Ferlin *et al.*, 2006) and one with undervirilization (Hiort *et al.*, 1996). Patient 22 presented a new p.Ala45Gly mutation.

The association of low basal T and high basal LH levels in patient 25 was discordant with his pubertal status, which prompted us to conduct further endocrine investigations. Most steroid compounds were evaluated at baseline, as well as after an ACTH stimulation test (Table 3). We found a clear discrepancy between the high basal progesterone (5-fold above normal values) and the low basal and non-ACTH stimulated DHEA and cortisol levels. CYP17A1 gene sequencing revealed compound heterozygosity consisting of c.275C>A/WT in exon 1 (plus three known SNPs: rs762163, rs6162, rs6163, all heterozygous) and c.887C>T/WT in exon 4, respectively, leading to p.Pro35Thr (P35T) and p.Arg239Stop (R239X) in the P450c17 protein (Fig. 1). The karyotype was 46,XY.

In order to assess the functional consequences of the mutations, COS1 cells were transfected with the expression vector pcDNA3 containing either wild-type or mutant *CYP17A1* cDNA.

When expressed in the COS1 cells, the mutant protein bearing the premature stop codon R239X showed a complete loss of 17α-hydroxylase and 17,20-lyase activity. In contrast, compared with the wild-type protein, the mutant P35T seemed to retain 15–20% of 17α-hydroxylase and about 8–10% of 17,20-lyase activity at a substrate concentration of 1.0 μmol/L (Fig. 2A). To further analyze the molecular mechanism of the complete loss of function in the nonsense mutations, we explored the possibility of nonsense-mediated mRNA decay, NMD, by stabilizing mRNA through the translation inhibitor cycloheximide, as there is evidence that some mRNA molecules with a premature stop codon are able to avoid detection and decay (Nagy & Maquat, 1998; Inacio *et al.*, 2004). However, the normal RNA (WT) was readily detectable, whereas the RNA derived from the R239X vector was nearly undetectable and could not be stabilized by cycloheximide (Fig. 2B). This suggests that NMD, and not the production of a truncated protein, was the reason for the loss of function.

We thus investigated this group of 25 adolescent males with P/PPG followed at the Pediatric Endocrine Clinic of the Montpellier University Hospital for the last 3 years. Among these patients, 18 (72%) were diagnosed with 'idiopathic' pubertal gynecomastia. Three patients presented Klinefelter syndrome, three a PAIS, and the last showed a 17α-hydroxylase/17,20-lyase deficiency. Genetic/hormonal causes therefore concerned 28% of our patients (95% confidence interval: 10–47).

DISCUSSION

Conversely to prepubertal gynecomastia, which is usually related to estrogen-producing adrenal or testicular tumors, pubertal gynecomastia is common, most often idiopathic, and regresses within a few months to 2 years (Biro *et al.*, 1990; Abaci & Buyukgebiz, 2007). Nevertheless, drug use, including anabolic steroids, and endocrine disorders should be considered when it is prominent (>Tanner B3) and/or persistent (>2 years) (Abaci & Buyukgebiz, 2007; Ma & Geffner, 2008).

Once drug involvement has been excluded, any endocrine disorders that result in an estrogen/androgen imbalance should be considered. An excess of estrogen production or a decrease in the production or action of androgens should be sought. Hyperestrogenism may result from excessive tumoral testicular or adrenal estrogen production. In addition, peripheral estrogen production may also be related to aromatase excess syndrome (Fukami *et al.*, 2011).

Regarding the reduction in T production, hypo- or hypergonadotropic hypogonadism may be involved in the development

of gynecomastia (Chan *et al.*, 1999; Narula & Carlson, 2007; Ma & Geffner, 2008). In addition, pubertal gynecomastia has been reported as one of the clinical expressions of PAIS (Sultan *et al.*, 2013).

Over the past several years, the hypothesis has emerged that environmental endocrine disruptors play a role in the development of pubertal gynecomastia through their agonistic action on ER receptors, as well as antagonistic effects on androgen receptors (Kalyan, 2007; Durmaz *et al.*, 2010).

Exceptional reports of gynecomastia associated with rare forms of congenital adrenal hyperplasia, including 3-β-hydroxysteroid dehydrogenase (Cavanah & Dons, 1993), 11-β-hydroxylase (Hochberg *et al.*, 1991) and, more recently, 21-hydroxylase deficiency (Wasniewska *et al.*, 2008) are still not totally explained.

The high percentage of 'idiopathic' gynecomastia in our cohort is in agreement with the literature on males at this age (Ma & Geffner, 2008; Atabek, 2013). It is nevertheless notable that 7/25 (28%) showed a genetic disorder. Even though this estimation is not precise because of our small sample size (95% confidence interval: 10–47), it suggests that a genetic etiology should be considered in such patients.

The diagnosis of Klinefelter syndrome in the pubertal period is unsurprising as most cases are diagnosed at this time (Pacenza *et al.*, 2012). A low T/E2 ratio is considered to play a key role in the promotion of breast development (Abdel-Razic *et al.*, 2012). Gynecomastia is reported in 50–70% of men with Klinefelter syndrome and is linked to an increased risk of male breast cancer (Swerdlow *et al.*, 2005; Narula & Carlson, 2007). Follow-up is thus necessary for adult patients.

Pubertal gynecomastia in patients with PAIS is well described, but it is usually associated with signs of undervirilization, such as micropenis, hypospadias, and cryptorchidism (Sultan *et al.*, 2013). The three patients with PAIS we report here were normally virilized, with their androgen resistance expressed exclusively through isolated pubertal gynecomastia. There have been several reports of pubertal gynecomastia revealing PAIS but in most cases undervirilzation or infertility was associated (Zenteno *et al.*, 2002; Lee *et al.*, 2015; Petroli *et al.*, 2014). Conversely, the follow-up of PAIS patients revealed the presence of P/PPG in 93% of the cases, but all these patients presented other signs of undervirilization (Hellmann *et al.*, 2012). The p.Ala646Asp mutation found in our two patients, 23 and 24, has already been reported in males with undervirilization (Hiort *et al.*, 1996) or infertility (Ferlin *et al.*, 2006), whereas the Ala45Gly mutation found in patient 22 has never been reported.

P/PPG has exceptionally occurred in adolescents with congenital adrenal hyperplasia because of 21-hydroxylase, 3-β-hydroxysteroid dehydrogenase or 11-β-hydroxylase deficiency (Ma & Geffner, 2008; Wasniewska *et al.*, 2008), but there are no data on the association of isolated P/PPG and 17α-hydroxylase/17,20-lyase deficiency. In agreement with Martin *et al.* (Martin *et al.*, 2003), who reported that basal progesterone measurement was a useful marker of 17α-hydroxylase/17,20-lyase deficiency, we suspected this deficiency in patient 25, even in the absence of corticosterone and 17hydroxypregnenolone values. To our knowledge, this is the first case of 17α-hydroxylase/17,20-lyase deficiency revealed by pubertal gynecomastia as the only sign of abnormality, with normal external genitalia and pubertal onset. Among the approximately 150 cases of 17α-hydroxylase/17,20-lyase deficiency reported in the literature, a 46,XY sex-reversal was predominant. Most cases have also been diagnosed in childhood or adolescence, through apparent mineralocorticoid excess

Table 3 Evaluation of steroid compounds at baseline, as well as after an ACTH stimulation test for patient 25

	Basal values	Values/ACTH
Cortisol (μg/dL)	5.6 (*N* > 12)	7.7
17-OHP (ng/mL)	3.8	4.5 (*N* < 10 ng/mL)
Prog. (nmol/L)	10.9 (*N*: 0.7–2)	10.8
DHEA (nmol/L)	1.8 (*N*: 14 ± 4.5)	2

The steroid compounds evaluated are the following: cortisol, 17 hydroxy progesterone (17-OHP), progesterone (Prog) and dehydroepiandrosterone (DHEA). The units are specified in brackets. N: normal references correspond to normal post-pubertal values.

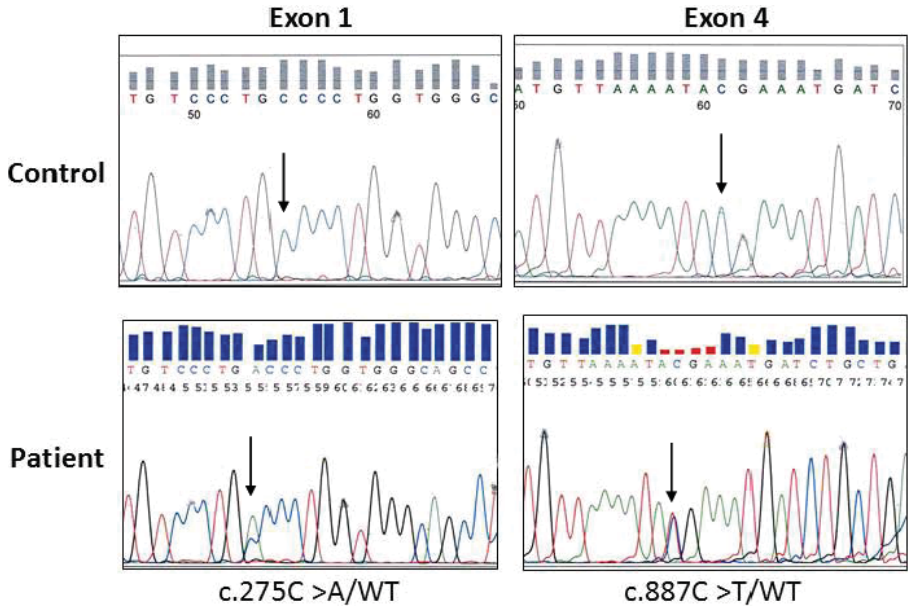
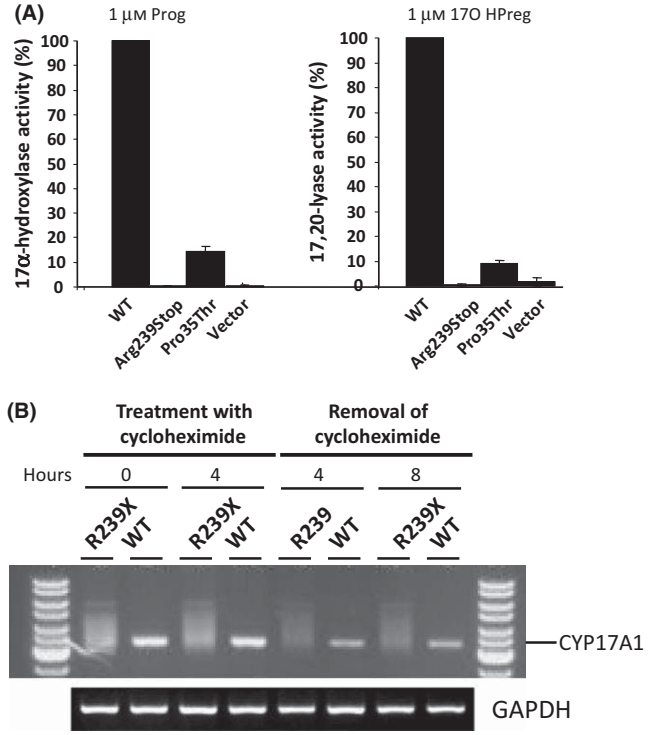


Figure 1 Sequence analysis of the CYP17A1 gene in control and patient 25. Arrows point out the amino acid substitutions in the patient compared with the control.

Figure 2 (A) Analysis of the 17α-hydroxylase and 17,20-lyase activity of the mutants P35T and R239X, in COS1 cells. (B) Analysis of the RNA stability of the R239X mutant carrying the premature stop codon to check for nonsense-mediated decay (NMD).



with salt retention and hypertension associated or not with delayed puberty (Miller, 2004). In addition, partial forms of 17α-hydroxylase/17,20-lyase deficiency in undervirilized 46,XY patients have been reported (Biason-Lauber *et al.*, 2000; Van Den Akker *et al.*, 2002; Martin *et al.*, 2003; Costa-Santos *et al.*, 2004; Idkowiak *et al.*, 2012). The absence of genital abnormality in our patient is unusual and indicates the likelihood of a slight enzymatic deficiency. Mild forms of 17α-hydroxylase/17,20-lyase deficiency have exceptionally been reported in 46,XY patients (New, 1970; Bosson *et al.*, 1988; de Lange & Doorenbos, 1990), two of whom were investigated for persistent gynecomastia (New, 1970; de Lange & Doorenbos, 1990). Conversely to the patient we report here, these patients presented no spontaneous pubertal development (de Lange & Doorenbos, 1990) or undervirilization (New, 1970). Although the direct relationship between the development of gynecomastia and the 17α-hydroxylase/17,20-lyase deficiency cannot be proven, it is likely that the androgen defect modifies the T/E balance, promoting breast development (New, 1970; de Lange & Doorenbos, 1990). In two 46,XY patients with very partial forms of 17α-hydroxylase/17,20-lyase deficiency (Bosson *et al.*, 1988; de Lange & Doorenbos, 1990), as in our patient, no hypertension was found, whereas slightly elevated blood pressure was noted in the patient reported by New (New, 1970).

Conversely to these three very slight enzymatic defects with a diagnosis only biologically established, the endocrine investigations in our patient (25) suggested a 17α-hydroxylase/17,20-lyase deficiency and this was confirmed by molecular analysis: we identified a double heterozygous mutation: p.P35T and p.R239X. To our knowledge, the p.P35T mutation of P450c17 has never been reported, whereas p.R239X was reported in a male patient with ambiguous genitalia who presented a p.P342T on the other allele (Ahlgren *et al.*, 1992). In order to confirm the causative role of these two mutations, functional analysis was performed. In transfected COS1 cells, the mutant protein bearing the premature stop codon R239X showed complete losses in 17α-hydroxylase and 17,20-lyase activity, as to be expected, whereas the mutant P35T seemed to retain 15–20% of 17α-hydroxylase and 8–10% of 17,20-lyase activity. The preservation of residual 17α-hydroxylase and 17,20-lyase activity is in agreement with the partial phenotype. The low T level related to a T biosynthesis defect, with a T/E2 imbalance, contributed to the breast development in our patient. After surgical removal of gynecomastia, adequate treatment with hydrocortisone and testosterone was introduced to prevent hypertension and achieve normal puberty. Despite oligospermia, sperm cryoconservation was performed.

Several study limitations must be acknowledged. First, as a result of the exploratory and descriptive nature of the study, the sample size was not sufficient to estimate precisely the prevalence of endocrine/genetic causes among the P/PPG population. Moreover, we cannot exclude a selection bias, as many adolescents do not consult for pubertal gynecomastia or only consult their general practitioner. The patients referred to our specialized center may therefore have been among the most serious cases.

In conclusion, our work showed that 28% of the cases of P/PPG in the adolescent males followed in our clinic had an endocrine/genetic cause. PAIS and 17α-hydroxylase/17,20-lyase deficiency may both be revealed by isolated P/PPG as the only clinical expression. Isolated P/PPG is not always a 'physiological' condition and should thus be investigated by endocrine and genetic evaluations, even though larger studies are needed to better determine the real prevalence of genetic defects in such patients.

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DISCLOSURE

The authors have nothing to disclose.

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