

## CASE REPORT

## A challenging case of primary amenorrhoea

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**SUMMARY**

The differential diagnosis of a girl presenting with primary amenorrhoea includes numerous conditions. Often, patients of 46XY disorder of sex development (DSD) are reared as girl and present with primary amenorrhoea. Their further evaluation to reach the final diagnosis is often a great challenge. In this article, we report a challenging case of 46XY DSD presented with primary amenorrhoea. Patient had spontaneous breast development which initially confused the diagnosis to complete androgen insensitivity syndrome. However, low testosterone suggested against this possibility and further evaluation revealed hormonal findings consistent with 17 $\alpha$  hydroxylase/17,20 lyase (CYP17A1) deficiency. Patient had 46XY karyotype and in consistence with hormonal findings patient was found to have a likely pathogenic homozygous c.1345C>T (p.Arg449Cys) variation in exon 8 of *CYP17A1*.

**BACKGROUND**

The differential diagnosis of a girl presenting with primary amenorrhoea includes numerous conditions.<sup>1</sup> Often, patients with 46XY disorder of sex development (DSD) are reared as girl and present with primary amenorrhoea.<sup>1</sup> Their diagnosis may go unnoticed unless karyotype testing is obtained. Further evaluation of a patient with 46XY DSD presenting with primary amenorrhoea to reach the final diagnosis is often a great challenge.<sup>2</sup> In this article, we discuss the challenges encountered in the evaluation of a patient with 46XY DSD presented with primary amenorrhoea.

**CASE PRESENTATION**

A 19-year-old reared as girl, presented with primary amenorrhoea. She had spontaneous breast development and except for the use of some unknown ayurvedic medication for 1 month for delayed menarche, no other hormonal supplements were used. She was born of a third-degree consanguineous marriage. She is the third in birth order and has two elder brothers and a younger sister (18 years); all of whom had normal pubertal development. There was no family history of ambiguous genitalia or hypertension. There was no history of chemotherapy or radiotherapy.

Patient weighed 55 kg and measured 156.4 cm (10th–25th centile on revised Indian academy of pediatrics (IAP) growth chart for girls and <3rd centile on revised IAP growth chart for boys). Mid-parental height for males was 163.9 cm and that for females was 150.9 cm. Upper segment to lower segment ratio was 0.85 with arm span height difference of 7.5 cm.

Blood pressure was 110/70 mm Hg. Sexual maturity rating revealed stage 5 of breast development and normal female external genitalia with two separate openings in the introitus (no clitoromegaly) with normal anogenital ratio (0.45). Pubic hair (P1), axillary hair and body hair were absent (figure 1A,B). Gonads were palpable over the respective superficial inguinal canals.

**INVESTIGATIONS**

Patient was evaluated with serum gonadotropins and total testosterone. Serum gonadotropins were elevated but serum total testosterone was low which was confirmed on repeated testing in multiple samples. Serum oestradiol was 34.64 pg/mL.

Ultrasound pelvis revealed no female internal genitalia and confirmed the testes-like structures in bilateral superficial inguinal rings. Further hormonal evaluation revealed undetectable androstenedione and very low dehydroepiandrosterone sulfate (DHEAS) with elevated 17-OH progesterone. Serum progesterone was high whereas serum antimüllerian hormone (AMH) was slightly higher. Serum 08:00 hours cortisol was normal with inadequate response to adrenocorticotrophic hormone (ACTH) stimulation. Serum potassium and plasma renin activity were normal (table 1).

Karyotype was 46XY. Clinical exome analysis revealed homozygous c.1345C>T (p.Arg449Cys) variation in exon 8 of *CYP17A1* which was confirmed with Sanger sequencing. This variant has not been reported in the 1000 genomes and has a minor allele frequency of 0.002% and 0.045% in the Exome Aggregation Consortium database. The *in silico* predictions of the variant are probably damaging by Polymorphism Phenotyping v2 and damaging by Sorting Intolerant from Tolerant, likelihood ratio test and Mutation Taster2. The reference codon is conserved across mammals. No significant variations were found in genes coding for p450 oxidoreductase, cytochrome b5 or androgen receptor.

**TREATMENT AND OUTCOME**

The patient underwent bilateral gonadectomy which was consistent with testes having Leydig cell hyperplasia (figure 1C). Genital examination under general anaesthesia revealed short, blind vagina. There were no perioperative complications. The patient was initiated on estradiol valerate (2 mg/day). Six months later, the patient was advised regular self vaginal dilatation.



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**Figure 1** Photograph depicting spontaneous Tanner stage 5 breast development (A), absent pubic hair (B) and histopathology showing a testis with Leydig cell hyperplasia and seminiferous tubules predominantly containing Sertoli cells (C).

## DISCUSSION

We report a case of *CYP17A1* deficiency which was initially confused with complete androgen insensitivity syndrome (CAIS) due to a history of spontaneous breast development which was Tanner stage 5.<sup>13</sup> This observation in our case suggests that the history of spontaneous breast development, even until stage 5, should not negate the possibility of *CYP17A1* deficiency. She also had detectable serum oestradiol levels which might be responsible for spontaneous breast development. However, this finding remains unexplainable in *CYP17A1* deficiency especially with very low DHEAS and undetectable androstenedione as seen in our patient.<sup>3</sup>

In fact, due to a history of spontaneous breast development, in conjunction with absent P1, she was suspected to have CAIS. Surprisingly, her serum total testosterone (9 ng/dL, 28 ng/dL, 37 ng/dL) levels were documented to be low at multiple times

which suggested against CAIS. Complete gonadal dysgenesis was unlikely in view of palpable gonads and absent müllerian structures. Hence, she was suspected to have either testosterone biosynthetic defect or CAIS with acquired Leydig cell damage. Testing for testosterone precursors revealed elevated progesterone, mildly elevated 17-OH progesterone with very low DHEAS and undetectable androstenedione which suggested a likely defect in 17 $\alpha$  hydroxylase/17,20 lyase (2,3). Although normotension, normokalemia and normal plasma renin activity favoured isolated 17,20 lyase deficiency, higher elevation of progesterone than 17-OH progesterone and inadequate cortisol response to ACTH also suggested the possibility of concomitant mild 17 $\alpha$  hydroxylase deficiency.<sup>3</sup> P450 oxidoreductase deficiency or cytochrome b5 mutations may lead to functional defects in combined 17 $\alpha$  hydroxylase/17,20 lyase or 17,20 lyase alone respectively. Hence, mutations in these may produce similar biochemical findings and hence, were considered in the differential diagnosis.<sup>4</sup>

To differentiate among these causes and obtain definitive genetic diagnosis, clinical exome sequencing was ordered which revealed most likely pathogenic variation in *CYP17A1*. Previously few cases of 17 $\alpha$  hydroxylase deficiency have been reported from India.<sup>5-9</sup> However, there is only one report of two siblings with proven *CYP17A1* mutation.<sup>9</sup> Ours is the second report of genetically proven *CYP17A1* deficiency from India. Previously R449C mutation in exon 8 has been reported in two patients from China but in compound heterozygous state with Y329K and R96Q.<sup>10</sup> Ours is the first case in the world's literature with homozygous R449C variation. Pathogenicity of Arg449Cys variation in *CYP17A1* is not yet proven. In addition to the electrostatic charges at Arg-347, and Arg-358, that at Arg-449 is also essential for binding of the enzyme with cytochrome b5 with *CYP17A1* for its lyase activity.<sup>11 12</sup> Mutations at these sites are associated with preserved 17 $\alpha$  hydroxylase activity but significantly reduced 17, 20 lyase activity.<sup>11 12</sup> Change of a polar amino acid (Arginine) to a non-polar one (Cysteine) may alter the electrostatic charges. Though, no data on the enzyme activity of R449C mutation in *CYP17A1* enzyme activities for different base changes at codon 449 have been reported. p.Arg449Ala is characterised by very low lyase activity but near normal hydroxylase activity whereas p.Arg449Lys mutation had 72% hydroxylase activity and 26% lyase activity.<sup>12</sup> An activity profile similar to p.Arg449Lys mutation might have led to the combined deficiency but with no hypertension in our patient.

**Table 1** Hormonal levels in blood and their comparison with normal reference ranges for age-matched males

	Observed value	Reference range
Follicle stimulating hormone (mIU/mL)	45.4	1.2–15.8
Luteinising hormone (mIU/mL)	53.2	1.3–9.6
Total testosterone (ng/dL)	27, 9, 36	280–1100
Oestradiol (pg/mL)	34.64	10–40
Androstenedione (ng/mL)	<0.3	0.4–1.5
Dehydroepiandrosterone sulfate ( $\mu$ g/dL)	15.5	61.2–493
17 $\alpha$ -OH progesterone (ng/mL)	13.67	0.32–1.47
Progesterone (ng/mL)	4.5	<0.2
8:00 hours cortisol ( $\mu$ g/dL)	11	7–25
ACTH stimulated cortisol ( $\mu$ g/dL)	13.1	>18
Anti-müllerian hormone (ng/mL)	21	0.7–19
Potassium (mEq/L)	4.1	3.5–5
Plasma renin activity (ng/mL/hour)	2.1	0.6–4.3

## Learning points

- Evaluation of a patient with 46XY disorder of sex development presenting with primary amenorrhoea is often a great challenge, especially to reach the definitive diagnosis.
- Spontaneous breast development, though extremely rare, may occur in patients with 17 $\alpha$  hydroxylase/17,20 lyase (*CYP17A1*) deficiency and its presence should not negate the diagnosis of *CYP17A1* deficiency.
- We report the first case in the world's literature of likely pathogenic homozygous R449C variation in *CYP17A1*.

**Contributors** Patient was evaluated by VS, SA and CS. RR did the bilateral orchidectomy. VS drafted the manuscript; all others critically reviewed and approved the manuscript.

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