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CASE REPORT

A rare enzymatic defect, true isolated 17,20-lyase deficiency leading to endocrine disorders and infertility: case report

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ABSTRACT

The cytochrome P450 17A1 catalyzes the formation of 17-hydroxysteroids and 17-ketosteroid. Most defects in CYP17A1 impair both enzymatic activities and cause a combined 17α-hydroxylase/17,20-lyase deficiency, which impairs hormone production (cortisol and sex steroids), sexual development, and puberty. Isolated 17,20-lyase deficiency is usually defined by evidently normal activity of 17α-hydroxylase with a dramatic decline of 17,20-lyase activity or complete inactivity. The changes in enzyme activity lead to a lack in the production of sex steroids with normal levels of glucocorticoid and mineralocorticoid hormones. A 24-years-old married woman, as a product of a consanguineous marriage, presented with infertility and a background marked by primary amenorrhea. Laboratory data showed low normal serum cortisol levels and low levels of 17-hydroxyprogesterone. Also, her adrenal androgens were low but estradiol was normal. The chromosomal investigation uncovered a male karyotype of 46, XY. These clinical and laboratory evidence confirm the determination of an isolated 17,20-lyase deficiency in a genotypic male.

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Infertility; adrenal hyperplasia; isolated 17,20-lyase deficiency; amenorrhea; gonads

Introduction

17 alpha(α)-hydroxylase/17,20-lyase deficiency (17OHD) is a condition that affects the activity of gonads (ovaries in females and testes in males) and the adrenal glands (graphical abstract) [1,2]. 17 alpha(α)-hydroxylase (17α OH) and 17,20-lyase activities are catalyzed by microsomal cytochrome P450c17 which is encoded by a single gene, CYP17A1 gene, located on chromosome 10q24-q25 [3]. 17OHD is one of a group of disturbances, known as congenital adrenal hyperplasia, that impairs hormone production and impair sexual development and puberty [4]. Hormone imbalances lead to the characteristic signs and symptoms of abnormal sexual development. The intensity of the features varies [5]. Two types of the condition are recognized: complete 17OHD, which is more intensive, and partial 17OHD, which is typically less severe. Disruption of sexual development affects males and females differently. The interesting diversity of this event appeared in patients with an isolated 17,20-lyase deficiency (ILD). In this very rare defect due to the fact that adrenal 17α-hydroxylase enzyme activity remains largely intact, whereas 17,20-lyase activity is completely or partially lost, the patient usually does not develop a clinical sign of hypertension. In ILD patients, the activity of the hydroxylase enzyme component leads to adequate cortisol production, and hence there is no increase in the production of mineralocorticoids, including corticosterone and deoxycorticosterone. Instead, in isolated 17,20-lyase activity defect, the synthesis of sex hormones degrades to a very low level, leading to main disorders in sexual maturity (pubarche) and ambiguous genitalia in 46, XY patients. The production of fetal testosterone hormone in the testes, which is essential for male sexual differentiation, requires activity of the 17,20-lyase (desmolase) enzyme [6,7]. Clinically reported cases of patients with putative isolated ILD are very rare [5]. In this case study, the clinical status of a patient with a diagnosis of isolated ILD with retained 17α-hydroxylase activity but minimal 17,20-lyase activity has been reported.

Case description

An Iranian married woman (age, 24 years) was referred to the Department of Endocrinology and Female Infertility by a gynecologist in Al-Zahra Hospital (Isfahan, Iran). She presented with primary amenorrhea and evidence of infertility. The patient had fully developed breasts (Tanner stage 5) with thin and fluffy pubic and axillary hair. She had no previous history of diagnosed mental illness, specific medical diseases or surgery. The patient presented with normal female sex characteristics, primary amenorrhea, no hypertension, and no hypokalemic periodic paralysis. Physical examinations revealed that the patient was relatively tall and thin, with a height of 172 cm, a weight of 70 kg and a body mass index of 23.7 kg/cm^2. Her blood pressure was normal (120/70 mmHg). Sexual maturation was assessed based on breast and pubic hair development. In this case, the external genitalia was phenotypically female and, the scores of breast and pubic hair development were B5P2 according to Tanner's classification. Cardiovascular examination revealed normal heart sounds with

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regular rhythm and normal muscular power. Further laboratory investigation on a number of parameters including white blood cell count (WBCs), platelet count, hemoglobin (Hgb), routine coagulation tests, blood biochemistry, and urine analysis revealed that the measured levels were within the normal range.

In the pelvic ultrasonography, the uterus and ovaries did not appear in their anatomical location but a region with tissue similar to a testis (11*18 mm) was seen in the right inguinal area. The pelvic magnetic resonance imaging (MRI) demonstrated oval soft tissue (15*10 mm) in the superior part of the left inguinal canal and oval tissue with 19*11 mm in size in the middle of the right inguinal canal that was suggestive for testes. The pituitary gland was normal. There was no evidence of uterus and ovary in the pelvic cavity (Figure 1).

After finding testes in the bilateral inguinal site, she had been suggested orchiectomy with coverage of corticosteroid. Three months after orchiectomy in renewed lab data, the estradiol level fell down and gonadotropins were more elevated, so we presumed the source of estradiol was testes, because after orchiectomy estradiol declined.

In the hormonal analysis, the serum cortisol level was low (6.8 µg/dl) with ACTH in the upper limit of the normal range (64.55 pmol/l). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were 16.3 (mIU/ml) and 65 (mIU/ml), respectively. Estradiol was in the normal range but total and free testosterone were low. In addition, the patient presented with low 17α-hydroxyprogesterone level (the precursor of cortisol), and low levels of adrenal androgens (dehydroepiandrosterone-sulfate (DHEA-S) and androstenedione) (Table 1).

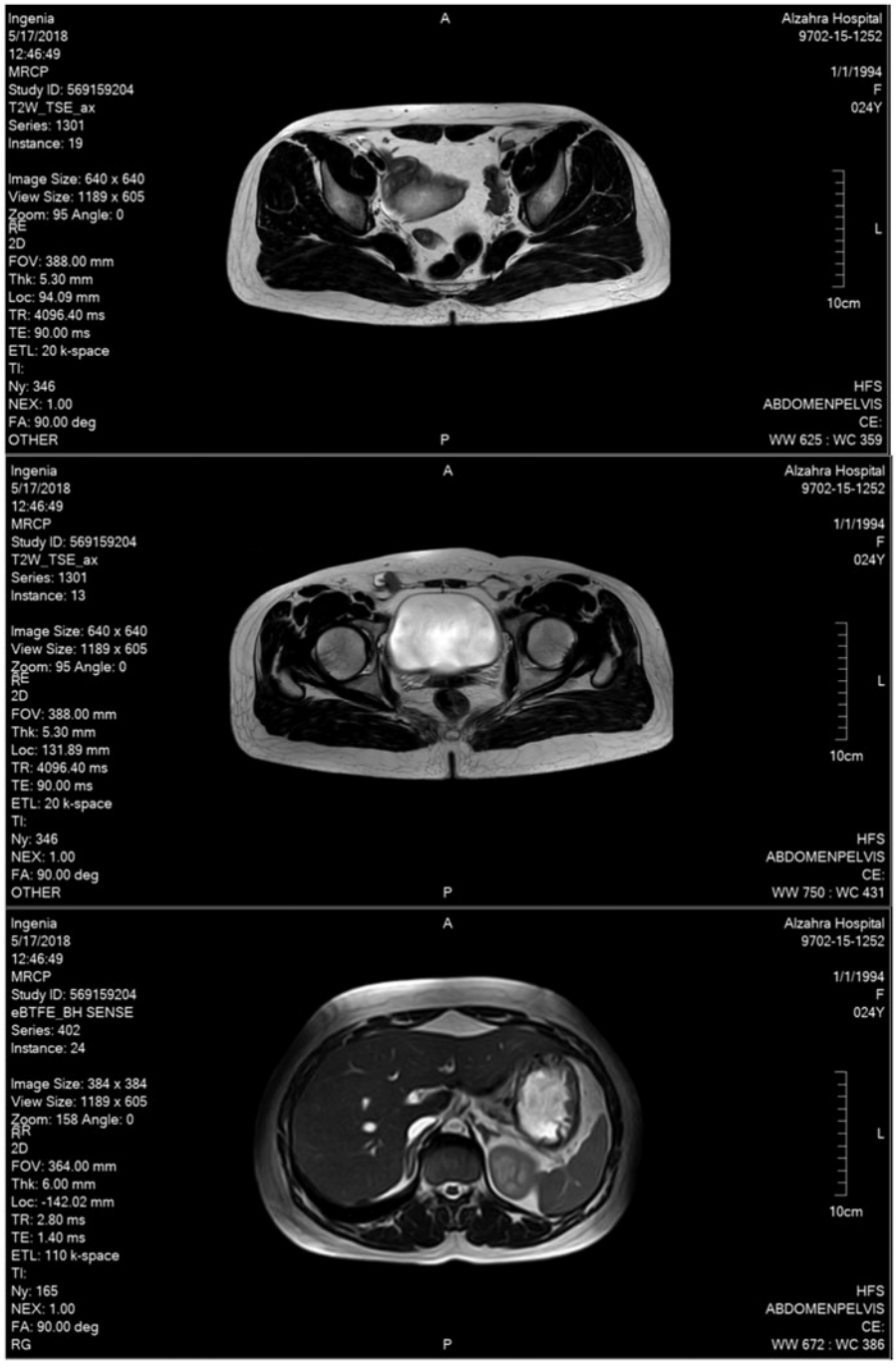

Figure 1. Magnetic resonance imaging (MRI) slides represent of the area being examined. The name and surname of the patient have been removed from the slides according to the ethical principles.

Table 1. Serum hormone levels before and after orchiectomy.

Plasma steroid/pituitary hormone	First visit	After orchiectomy	Reference range
FSH (mIU/ml)	16.3	74.8	Follicular phase:2.5–10.2 Luteal phase:1.5–9.1 Post menopausal:23–116.3 Males:1.4–18.1
LH (mIU/ml)	65	55.68	Follicular phase:1.9–9.2 Luteal phase:1.3–10.8 Post menopausal:15.4–53.3 Males:2.8–6.8
Estradiol (pg/ml)	23	1.8	<10 (pg/ml)
17 0H-Progesterone (ng/ml)	5.60		0.35–4.13
Testosterone (ng/ml)	0.05		Females:0.14–0.76 Male:2.41–8.27
Prolactin (ng/ml)	7.285		Females: Non pregnant:2.8–29.2 Pregnant:9.7–208.5 post menopausal:1.8–20.3 male:2.1–17.7
DHEA-S (micg/dl)	10.04		25.9–460.2
Androstenedione (nmol/l)	2.1		3.2–6.9
17α-hydroxyprogesterone (ng/ml)	18.4		Men:0.2–2.3 Females: Follicular phase:0.2–1.3 Luteal phase:1–4.5 menopausal:0.2–0.9
ACTH (pmol/l)	64.55		7.2–63.6
Cortisol (μg/l) 8:00 a.m	6.89		
Cortisol after DST 4h (μg/l)	12.06		
Plasma Renin activity (ng/ml/h)	5.29		0.06–4.69
Plasma Renin (pg/ml)	48.5		Supine:4.2–59.7 Upright:5.3–99.1
Aldosterone (ng/dl)	35.4		Upright:3.7–31.0 Supine:3.7–43.2
Aldosterone/Renin ratio	0.7		0.45–49.65

Moreover, her 17-year-old sister had primary amenorrhea with incomplete sexual maturation. So we evaluated her sister too. In physical examination, she had no secondary sexual development. Her Tanner stage was B1P1 and laboratory data revealed hypergonadotropic hypogonadism with low estradiol, low testosterone, and 17-OH progesterone. Electrolytes were normal. In pelvic sonography, there was no evidence of uterus and ovaries. In karyotype examination, her genotype was reported 46,XY.

The bone mass measurement was done by dual-energy X-ray absorptiometry (Norland) on the lumbar spine (L1-L4), hip and, wrist region. In the case of wrist bone scan, the epiphyseal cartilage ossification was complete. Bone age assessment was performed by taking a single X-ray radiograph of the left wrist, hand, and fingers. Bone age was equal to that of an adult. In the case of BMD there was no osteoporosis by comparing the *Z*-score, but osteopenia was seen in the lumbar region (Figure 2, Table 2).

At the next step, we performed a chromosome study. The chromosomal analysis revealed 46,XY male karyotype (Figure 3). Considering the clinical, imaging and laboratory data, we presumed she suffered a type of congenital adrenal hyperplasia, so we performed CYP17A1 sequence genetic analysis on the patient and her parents (Table 3). To this end, the genomic DNAs were extracted from the peripheral blood leukocytes and exon 6 of CYP17A1 gene was amplified by polymerase chain reaction (PCR). Amplifications were also sequenced. Direct sequencing of PCR products revealed that the patient harbored a homozygous mutation in the CYP17A1 gene: c. 1039 C > T, leading to amino acid alterations p.R347C. Analysis of DNA from the patient's parents demonstrated that they are heterozygote for (c.1039C > T (p.R347C)) mutation (Table 3).

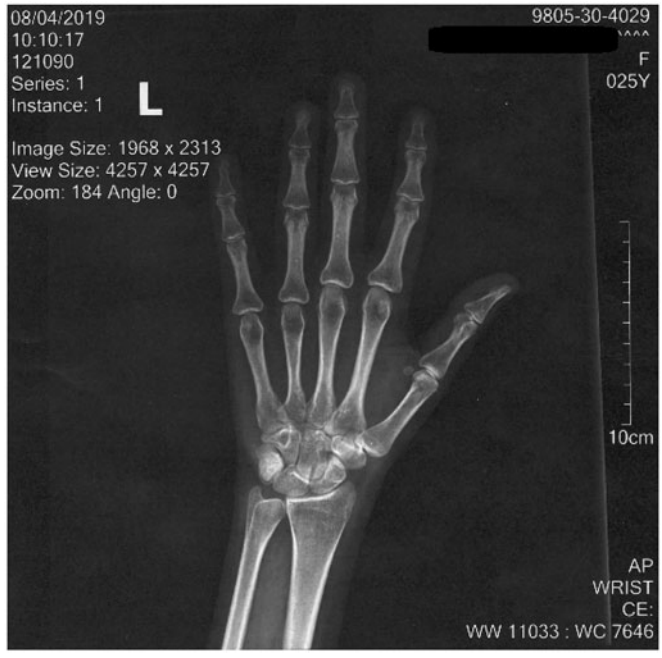

Figure 2. Single X-ray radiograph of the left wrist, hand, and fingers. Bone age assessment helps to estimate the maturity of skeletal system.

Discussion

Isolated ILD, as a rare autosomal recessive genetic disorder, is associated with endocrine system disruption. It results in the androgen and estrogen sex steroids deficiency and clinically manifests normal phenotype of female genitalia at birth,

Table 2. The bone mineral density test results; the bone mass measurement was done by dual-energy x-ray absorptiometry on lumbar spine (L1–L4), hip and wrist region.

	BMD	Young adult		Age matched	
Region	g/cm^2	%	*T*-Score	%	*Z*-Score
Hip (neck)	0.9070	91.9	−0.68	94.7	−0.43
Spine (L1-L4)	0.8746	80.5	−−1.33	81.4	−1.25
L-Forearm	0.2915	91.4	−0.57	91.2	−0.58

Region	BMD (g/cm^2)	BMC (g)	Area (cm^2)	Length (cm)	Width (cm)	*T*-score	Young ref. (%)	*Z*-score	Age match (%)
Left hip on: 08/04/2019 09:00:07 AM STD CVs for Neck BMD: 1.2 BMC: 1.7 See Guide for other CVs. 1.0 mm × 1.0 mm, 90 mm/s, 9.00 cm, Rev. 4.5.0/2.3.3 Calib. 07/29/19 S/N 20257									
Fem Neck	0.9070	4.657	5.134	1.50		−0.68	91.9	−0.43	94.7
Troch	0.6264	9.267	14.79			−1.47	79.6	−1.21	82.6
Wards Area	0.6335	0.6335	1.000	1.00	1.00	−1.74	74.4	−1.41	78.2
Totals BMD	868.5 mg/cm^2	30844 mg	35.51			−0.71	90.8	−0.70	91.0
AP spine on: 08/04/2019 08:54:23 AM STD CVs for L1-L4 BMD: 1.0 BMC: 1.5 See Guide for other CVs. 1.5 mm × 1.5 mm, 130 mm/s, 13.65 cm, Rev. 4.5.0/2.3.3 Calib. 07/29/19 S/N 20257									
L1 - L4	0.8746	60.24	68.87	14.1	13.7	−1.33	80.5	−1.25	81.4
L1	0.8297	12.58	15.16	3.15	13.7	−1.29	80.6	−1.32	80.2
L2	0.8950	14.77	16.50	3.60	13.7	−1.24	81.6	−1.22	81.8
L3	0.9220	17.57	19.06	3.90	13.7	−1.13	82.7	−1.09	83.2
L4	0.8439	15.32	18.15	3.45	13.7	−1.41	78.0	−−1.30	79.4
Totals BMD	941.2 mg/cm^2	64825 mg	68.87	14.1	13.7	−1.33	80.5	−1.26	81.4
Left forearm on: 08/04/2019 08:47:25 AM STD CV for Proximal Radius BMD: 0.9 See Guide for other CVs. 1.0 mm × 1.0 mm, 8 mm/s, 8.00 cm, Rev. 4.5.0/2.3.3 Calib. 07/29/19 S/N 20257									
Dist. R + U	0.2915	1.280	4.392	1.00	8.00	−0.57	91.4	−0.58	91.2
Prox. R + U	0.7056	1.559	2.210	1.00	8.00	−2.00	82.5	−2.05	82.1
Prox. R	0.6999	0.8551	1.222	1.00	8.00	−1.98	82.5	−2.01	82.3

irrespective of the genetic sex, and usually presents at puberty with primary amenorrhea with absent or disturbed pubertal development of secondary sexual characteristics in both 46,XY and 46,XX genotypes, leading to a somewhat childish appearance in adolescence (if left untreated) [8]. Regarding the lack of impairment of 17α-hydroxylase activity, cortisol deficiency or mineralocorticoid excess have not been reported in IDL patients, and for this reason, it does not lead to hypertension or congenital adrenal hyperplasia, which are mainly observed in a classic, combined CYP17A1 deficiency [2].

To date, only a small number of confirmed cases due to mutations (mainly missense mutations) in the CYP17A1 gene have been reported to cause true isolated ILD [9].

The clinical symptoms of isolated ILD, in affected 46,XY individuals are related to male pseudohermaphroditism with feminized, ambiguous, or moderately underdeveloped external genitalia, as a consequence of impaired synthesis of androgens (testosterone) in the testes. In 46,XY cases of partial ILD, because of low levels of androgen, which leads to a lack of suppression of estrogen synthesis, low virilization and gynecomastia up to Tanner stage V are evident clinical events [8].

In females, amenorrhea or, in cases of only partial deficiency, only irregular menses, and enlarged cystic ovaries are seen. Moreover, in both sexes, such symptoms as hypergonadotropic hypogonadism, delayed, impaired, or fully absent adrenarche and puberty with an associated reduction in or complete lack of development of secondary sexual characteristics, impaired fertility or complete sterility, tall stature, eunuchoid skeletal proportions, delayed or absent bone maturation, and osteoporosis are presented [10,11].

Males and females may be treated with hormone replacement therapy (i.e. with androgens and estrogens, respectively), which will result in normal sexual development and resolve most symptoms. In the case of 46,XY (genetically male), individuals who are phenotypically female and/or are identified as the female gender, should be treated with estrogens instead [12]. Removal of the undescended testes should be performed in 46,XY females to prevent their malignant degeneration, whereas in 46,XY males surgical correction of the genitals is generally required, and, if necessary, an orchiopexy may be performed as well. Namely, in genetic females presenting with ovarian cysts, GnRH analogs may be used to control high FSH and LH levels if they are unresponsive to estrogens [12].

The case report describes an isolated ILD patient with 46,XY disorder of sex development caused by the homozygous mutation in the CYP17A1 gene: c. 1039 C > T, leading to amino acid alterations p.R347C. In this case, the phenotype was female with puberty stage B5P2 but she had primary amenorrhea. Unlike the case report that was presented by Costenaro et.al. [13], breast development in our case was normal at stage 5. In history, she had no hypertension or hypokalemia, but in the case with combined 17α-hydroxylase/17,20-lyase deficiency reported by Papi et al. [14], the patient's past history consisted of long-lasting high blood pressure and hypokalemia, due to the accumulation of mineralocorticoid precursors.

In laboratory data, the estradiol was normal but LH and FSH elevated, the testosterone level was low too. However, in the cases that were reported by Costenaro [13] and Papi [14], the estradiol was low. In physical examination, the blood pressure was normal with normal serum electrolytes. Although basal serum cortisol was low (6.8 μg/ml) and did not pass the tetracosactide test, the patient had no history of the adrenal crisis. In the abdomen and pelvic MRI, there was no adrenal hyperplasia too (Figure 1). The karyotype examination revealed 46,XY genotype. Then in genetic evaluation homozygous mutation in the

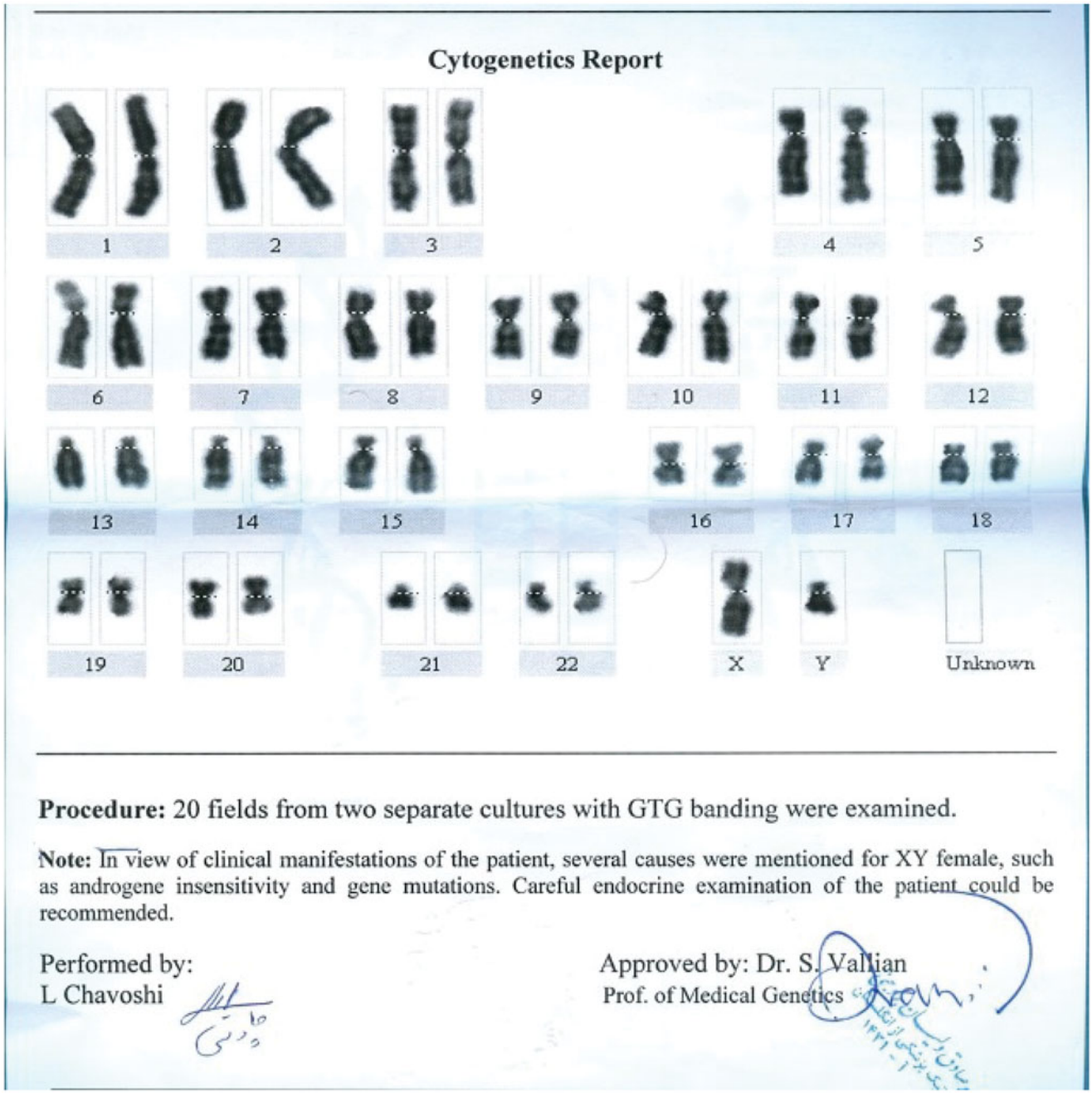

Figure 3. Cytogenetics report; the patient is apparently (phenotypically perfect female) female with chromosomal constitution of 46, XY.

Table 3. Identification and/or association of mutations confirmed by the methods below.

Techniques used				
Name	Phenotype	Exon	Genotype	Sequencing
Her father	Carrier	6	CAH (NM_000102.3) c.1039C > T(p.R347C) / N	–
Her mother	Carrier	6	CAH (NM_000102.3) c.1039C > T(p.R347C) / N	–
Herself (case study)	Affected	6	CAH (NM_000102.3) c.1039C > T(p.R347C) / c.1039C > T(p.R347C)	✓

The above techniques were used for each of the above persons and the conclusion is that case study patient is homozygote for c.1039C > T(p.R347C) mutation. Her father and her mother are carriers of Congenital Adrenal Hyperplasia (CAH)(CYP17A1 gene) and they are heterozygote for c.1039C > T(p.R347C) mutation.

CYP17A1 gene: c. 1039 C > T, which leads to amino acid alterations p.R347C had been reported.

The breast enlargement may be due to gynecomastia or the effect of estradiol. As our knowledge, in the past case reports elevated estradiol with breast stage-5 had not been reported, this made our case special.

In conclusion, regarding the scarcity of isolated ILD and unusual clinical manifestations in our case such as lack of

hypertension, hypokalemia and normal estradiol level as well as the involvement of two family members, it was decided to report this very rare case.

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Compliance with ethical standards

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Isfahan The Endocrine and Metabolism Research Center of Isfahan University of Medical Sciences approved the study.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Disclosure statement

The authors declare that they have no conflict of interest.

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