·CASE ANALYSES· ·临床病例讨论·

DOI:10.11817/j.issn.1672-7347.2022.210401

Aromatase deficiency caused by mutation of *CYP19A1* gene: A case report

LI Hongli[1], FU Songbo[1], DAI Ruchun[2], SHENG Zhifeng[2], LIU Wei[2]

(1. Department of Endocrinology, First Hospital of Lanzhou University, Lanzhou 730000; 2. National Clinical Research Center for Metabolic Diseases; Department of Metabolism and Endocrinology, Second Xiangya Hospital, Central South University, Changsha 410011, China)

ABSTRACT Aromatase deficiency (AD) is a rare autosomal recessive genetic disease caused by loss-of-function mutations in aromatase gene (*CYP19A1*), leading to congenital estrogen deficiency syndrome. Both mothers of AD patients during pregnancy and female AD fetus show virilization, while male patients are usually diagnosed in adulthood due to continued height increase and metabolic abnormalities. In 2019, a patient with AD was admitted in the Second Xiangya Hospital. The patient was a 37-year-old adult male who continued to grow linearly after adulthood. His estradiol was below the measurable line, the follicle-stimulating hormone (FSH) increased, bone age delayed, epiphysis unfused, and the bone mass reduced. *CYP19A1* gene detection showed that c. 1093C>T, p. R365W was homozygous mutation. This disease is rare in clinic. Clinicians need to raise awareness of the disease for early diagnosis and treatment to improve the long-term prognosis of patients.

KEY WORDS aromatase deficiency; *CYP19A1* gene; estrogen deficiency

CYP19A1 基因突变致芳香化酶缺乏症 1 例

李红利[1]，傅松波[1]，戴如春[2]，盛志峰[2]，刘玮[2]

（1. 兰州大学第一医院内分泌科，兰州 730000；2. 国家代谢性疾病临床医学研究中心，中南大学湘雅二医院内分泌科，长沙 410011）

[摘 要] 芳香化酶缺乏症(aromatase deficiency，AD)是一种罕见的芳香化酶基因(*CYP19A1*)失活突变引起的常染色体隐性遗传病，导致先天性雌激素缺乏综合征。AD患者的母亲在妊娠期以及女性胎儿出生时均可出现男性化表

Date of reception: 2021-06-23

First author: LI Hongli, Email: lihl19@lzu.edu.cn, ORCID: 0000-0001-6858-9796

Corresponding author: LIU Wei, Email: liuwei_13510@163.com, ORCID: 0000-0001-5626-8434

Foundation item: This work was supported by the Natural Science Foundation of Hunan Province, China (2020JJ5816).

现，而男性患者通常在成年后因身高持续增长和代谢异常才得以诊断。2019年中南大学湘雅二医院收治了1例AD患者，该患者为37岁成年男性，成年后仍持续生长，其雌二醇低于可测线、促卵泡激素(follicle-stimulating hormone，FSH)升高、骨龄落后、骨骺未闭合、骨量减少，*CYP19A1*基因检测示c.1093C>T，p.R365W纯合子突变。该病临床较罕见，临床医生需提高对该病的认识，早期诊断和治疗，以改善患者的长期预后。

[关键词] 芳香化酶缺乏症；*CYP19A1*基因；雌激素缺乏

Aromatase deficiency is a very rare autosomal recessive inherited disease, resulting from the loss-of-function mutation of aromatase gene (*CYP19A1*) that failed to convert androgen into estrogen. High level of androgen and low level of estrogen lead to varying degrees of virilization in the mother during pregnancy and in the female fetus, and affect metabolism, bones, and reproduction in the subsequent growth and development stages of both sexes[1-2]. Until now, 44 cases (30 females and 14 males) of aromatase gene defects have been reported since the first case was published in 1991 in Japan[3-11], and only 5 cases derived from China[3, 5, 11]. Thirty-three kinds of deleterious mutations in *CYP19A1* coding genes have been discovered, including point mutations, deletions, insertions, duplications, inversions, and multiple splice site mutations[2-3, 9]. This paper reports a case admitted to the Second Xiangya Hospital of Central South University with the aim of raising awareness of the disease among clinicians.

1 Case presentation

A 37-year-old Chinese man, complained of continued increase of height after adulthood. He had grown about 20 cm in height and 40 kg in weight over the past 17 years, but did not pay attention until he started having pain in both knees since 2020. He was the only child, and his growth and development before puberty was not different from other children. His mother had facial acne when she was pregnant and was diagnosed as “pregnancy-induced hypertension” in her 5th month of pregnancy. His parents were not close relatives. His father is 162 cm and his mother is 159 cm in height. The family had no similar medical history and denied other family medical history. He was unmarried, had no child, and had regular sexual life with a female partner.

Physical examination showed that the patient was 192 cm in height (genetic height 167 cm), upper part 90 cm, lower part 102 cm, arm span 200 cm, weight 113 kg, BMI 30.65 kg/m^2, abdominal circumference 117 cm, waist-to-hip ratio 1.1∶1, blood pressure 138/79 mmHg (1 mmHg=0.133 kPa). Multiple parts of the skin showed acanthosis nigricans (Figure 1). He had bilateral genu valgum (Figure 2A), incompletely fused epiphyses (Figure 2B), osteopenia (Figure 2C) and the bone age was 17－18 years old (Figure 2D). Informed consent was obtained from patients for the use of all images in this article. Laboratory test showed that his follicle-stimulating hormone (FSH) was elevated and the estradiol was undetectable (Table 1), combined with higher LDL cholesterol level and lower HDL cholesterol level, elevated total Type I collagen N-terminal extension peptide and β-collagen special sequence level, impaired glucose tolerance level, and hyperinsulinemia. His hemoglobin A1c was 6.20% (Table 1). He suffered from severe steatohepatitis, with low bone mass and hypertension. The fat content was 36.7% (at the 94th percentile of males at the same age). Computer-aided sperm analysis (CASA) semen analysis showed decreased forward-moving sperm without other obvious abnormalities (Table 1).

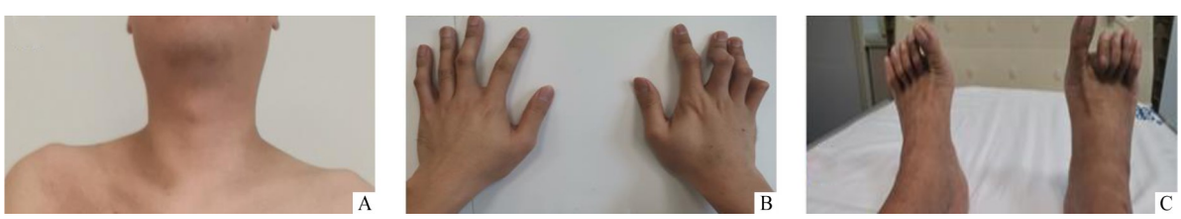

Figure 1 Acanthosis nigricans in multiple parts of the skin

Based on above evidences, the diagnosis was considered as aromatase deficiency. To confirm it, the whole genome DNA was extracted using peripheral venous blood after obtaining the informed consent from the patient and his parents. NM_000103 (*GRCh37*) gene, the sequence of the exon region (coding region) of *CYP19A1*, was directly sequenced and compared with the reference sequence. Gene sequencing revealed that the patient's *CYP19A1* gene c. 1093C>T (p. R365W) had a C→T mutation at 1 093 base pair (bp) in the 9th exon (Figure 3), causing tryptophan replaces arginine at position 365, which was a possible pathogenic variant. Homozygous variants were possible pathogenic variants according to the American College of Medical Genetics and Genomics (ACMG) guideline[12]. Different amino acid changes at the same site were detected in patients with aromatase deficiency (there was a G→A mutation at 1 094 bp in exon 9, resulting in glutamine replace arginine at position 365, and the aromatase activity of the mutant protein was 0.4% of that of the wild-type protein to support the functional importance of this region of the protein[13]). The mutation had not detected in the general population information database, so it was a new mutation. Bioinformatics softwares predicted that the variant was a pathogenic variant (Table 2). We used SWISS MODEL (https://www. expasy. org/resources/swiss-model) to predict 3D structure of the mutant protein to mimic the effect of the mutated region. As the result presented, the amine acid residue substitution caused change in local structure compared with wild-type structure (Figure 4). The patient's clinical manifestations were consistent with aromatase deficiency. Unfortunately, the determination of the aromatase activity of the mutant protein was not completed. As the diagnosis was aromatase deficiency, the patient was discharged from hospital after being supplemented with estrogen, vitamin D, and other related treatments. It was a pity that his condition was not followed up after his discharge from the hospital.

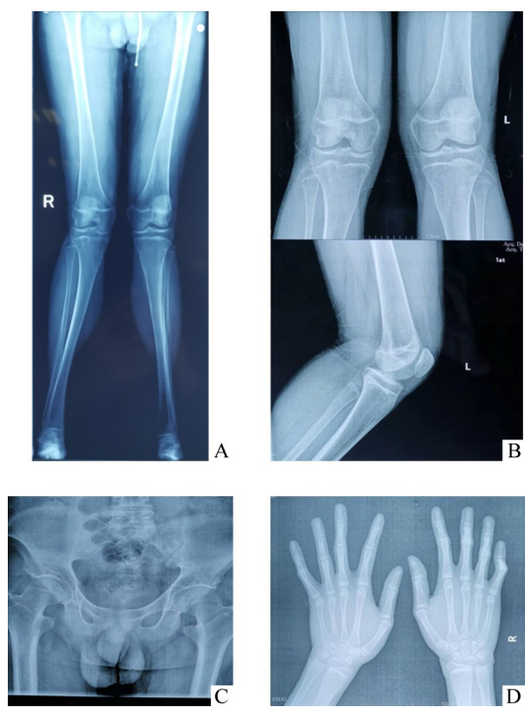

Figure 2 X ray of the patient with aromatase deficiency

A: Bilateral genu valgum; B: Incomplete fusion of the long bones of the upper and lower extremities; C: Bone loss; D: Bone age of 17−18 years.

Table 1 Test in the aromatase-deficient man

Variables	Values	Reference ranges
Total cholesterol/(mmol·L^{-1})	4.74	2.90−5.20
LDL cholesterol/(mmol·L^{-1})	3.47	<3.12
HDL cholesterol/(mmol·L^{-1})	0.83	>1.04
Triglycerides/(mmol·L^{-1})	1.38	<1.71
Estradiol/(nmol·L^{-1})	<0.04	0.04−0.53
Testosterone/(nmol·L^{-1})	21.96	8.40−28.70
Follicle-stimulating hormone/(U·L^{-1})	32.82	1.40−18.10
Luteinizing hormone/(U·L^{-1})	9.14	1.50−9.30
Alkaline phosphatase/(U·L^{-1})	<200	50−135
Osteocalcin/(ng·mL^{-1})	34	14−46
Total Type I collagen N-terminal extension peptide/(ng·mL^{-1})	132.20	9.06−72.04
β-collagen special sequence/(pg·mL^{-1})	963	47−783

Table 1 (to be continued)

Variables	Values	Reference ranges
25-hydroxyvitamin D/(nmol·L^{-1})	23	Deficiency <50; lack: 50−75; sufficient: 75−250
Hemoglobin A1c/%	6.20	3.90−6.10
OGTT		
Fasting plasma glucose/(mmol·L^{-1})	4.68	3.60−6.10
2 h postload glucose/(mmol·L^{-1})	9.29	<7.80
Fasting insulin/(mU·L^{-1})	102.4	6.4−15.0
2 h insulin/(mU·L^{-1})	108.0	6.4−15.0
Sperm concentration/(×10^{5}·mL^{-1})	75.2	≥15.0
Sperm vitality/%	47.2	≥40.0
Forward-moving sperm PR	19.30	≥32.00
Bone age/years	17−18	
Lumbar spine (L_{1-4}) Z-score (DEXA)		
L_1	−3.4	≥−2.0
L_2	−2.8	≥−2.0
L_3	−2.7	≥−2.0
L_4	−2.0	≥−2.0
Total	−2.2	≥−2.0
Femoral neck Z-score (DEXA)		
Neck	−2.0	≥−2.0
Troch	−2.8	≥−2.0
Inter	−2.0	≥−2.0
Total	−2.2	≥−2.0
Ward's	−2.4	≥−2.0

LDL: Low-density lipoprotein; HDL: High-density lipoprotein; OGTT: Oral glucose tolerance test; DEXA: Dual-energy X-ray absorptiometry; PR: Progressive sperm.

Table 2 Prediction results of the gene mutation

Tools	Scores	Reference values	Results
SIFT	0.0	0−0.05 damaging 0.05−1.00 tolerated	Damaging
Polyphen-2_HDIV	1.0	0.85−1.00 probably damaging 0.85−0.15 possibly damaging 0−0.15 benign	Probably damaging
Polyphen-2_HVAR	1.0	0.85−1.00 probably damaging 0.85−0.15 possibly damaging 0−0.15 benign	Probably damaging

SIFT: Scale invariant feature transform.

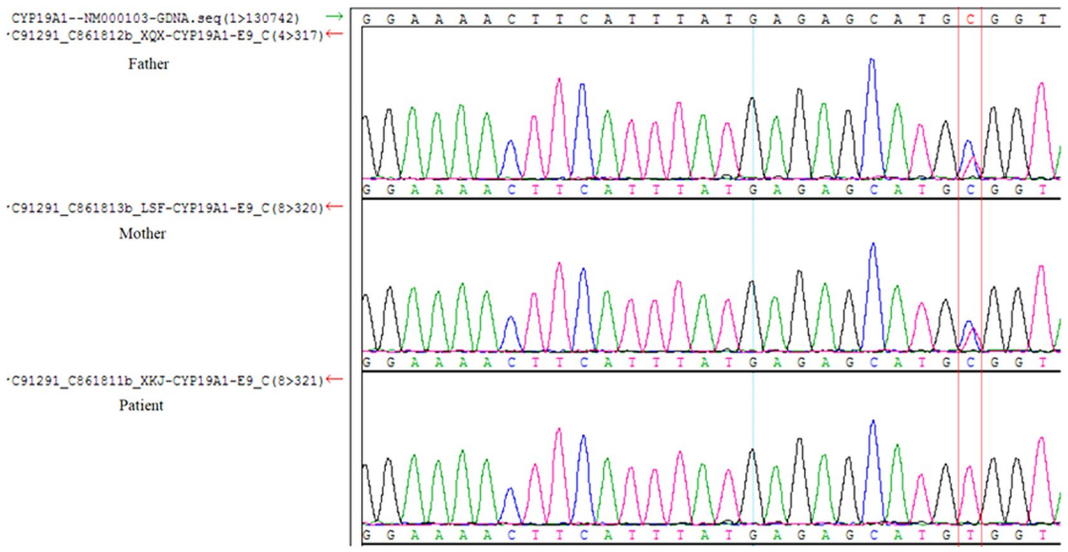

Figure 3 Genetic analysis of *CYP19A1* gene of the family

DNA sequence chromatogram of the proband compared with the reference. *CYP19A1* gene c.1093C>T (p. R365W) at 1 093 bp in the 9th exon (indicated by red frame).

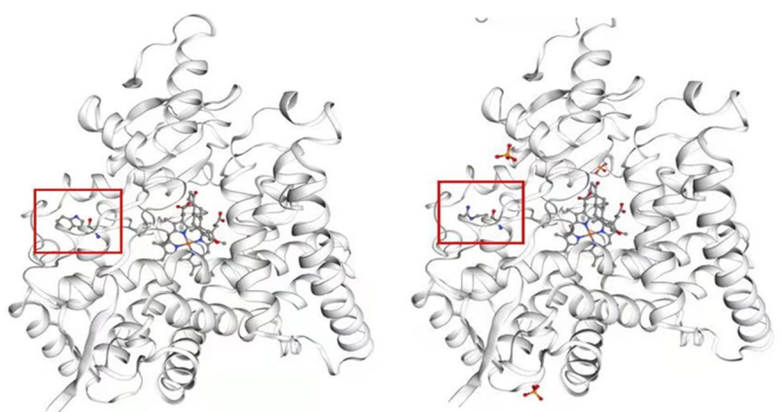

Figure 4 3D structure prediction of the mutation and wild-type proteins

2 Discussion

Aromatase is the key enzyme in the synthesis of human estrogen, and is the only cytochrome P450 enzyme that catalyzes androgens (C19 steroids) into estrogen (C18 steroids) in all vertebrates[2]. *CYP19A1* is located on chromosome 15q21.2 and consists of 10 exons. Exons 2 to 10 are coding ones, and exon 1 is related to tissue-specific gene expression[14]. Besides maintaining female secondary sexual characteristics, estrogen can also maintain bone mass, regulate lipoprotein synthesis, protect cardiovascular health, prevent urogenital atrophy, regulate insulin responsiveness, and maintain cognitive function[15]. Androgens in the fetal adrenal glands are the main source of circulating estrogen during pregnancy. Androstenedione, testosterone, and 16α-hydroxytestosterone cannot be converted into estrone, 17β-estradiol and 17β, 16α-estriol, respectively in the absence of placental aromatase, so a large amount of androstenedione and testis hormones are transferred to blood circulation of the mother and fetus[16].

Patients with aromatase deficiency in the absence of estrogen-induced adolescent epiphyseal fusion usually show continuous linear growth without accelerated puberty growth, tall stature, decreased bone density, delayed bone age, unfused epiphyses, eunuch-like bone ratio, extensive bone pain, and progressive genu valgum[11,16]. The effect of hormone changes on metabolism is similar to metabolic syndrome[17], including abdominal obesity, insulin resistance, acanthosis nigricans, non-alcoholic steatohepatitis, and

dyslipidemia[17-18]. Hyperinsulinemia is an important factor contributing to hyperandrogenism[19]. Furthermore, hyperandrogenism in turn aggravates insulin resistance[20], and insulin resistance and hyperinsulinemia can lead to the occurrence of characteristic acanthosis nigricans[21]. With high levels of androgens, the mother appears virilization (such as low voice, acne, hirsutism, and clitoral hypertrophy), gradually escalates[3], and then subsides 5 to 6 months postpartum. As long as 1% of the normal aromatase activity of placenta is sufficient to prevent maternal virilization during pregnancy, and not every mother will have virilization[2-3, 11]. Female aromatase deficiency patients will be born with pseudo-hermaphroditism and virilization of the external genitalia, and they will subsequently have delayed puberty, high gonadotropin sexual dysfunction, and polycystic ovary when they grow up[3]. About 70% of female patients have a severe degree of virilization, and will worsen with age[2]. Male aromatase deficiency patients are often diagnosed after puberty due to continuous height increase and metabolic abnormalities, which often leads to delay in diagnosis [age of diagnosis is (27.8±4.7) years], and have more obvious manifestations[2-3]. A small number of male patients may have genital and sperm damage. The clinical manifestations are affected by age, gender, and enzyme activity, and most of them will show varying degrees of improvement after treatment.

Due to the rarity of the disease, the reliability of diagnosis based on clinical data is poor. Genetic testing is required for patients with suspected clinical manifestations, medical history or examination. The DNA sequencing of 9 exons (E2 to E10) of *CYP19A1* should be performed, and the aromatase activity of the mutant protein is determined after transfection of the cells[18]. The treatment of aromatase deficiency is carried out in stages. The dosage of estrogen replacement therapy are mainly based on the actual situation. Researchers[23] speculated that females need low doses of 17β-estradiol (50 to 100 μg/d) in early childhood, and higher doses of estrogen (1.4 to 2.0 mg/d) in pre-puberty and adolescence. Males should take a small dose of estradiol [0.80 to 0.12 μg/(kg·d)] in early puberty, and gradually increase in the middle of puberty (16 to 17 years old)[18]. Low-dose estrogen for lifelong replacement therapy (25 to 50 μg/d of transdermal estradiol) is needed once epiphysis is closed[18]. Treatment should be adjusted according to the treatment outcome, gender, age, and stage of growth and development. At the same time, calcium and vitamin D are needed to achieve a synergistic effect on the bones. Lifestyle changes (such as quitting smoking, monitoring blood pressure) are needed to help prevent the exacerbation of cardiovascular disease. Other skeletal abnormalities (such as genu valgum) can be surgically corrected[17].

This patient was a 37-year-old male. His mother had facial acne during pregnant. He was asymptomatic in infancy and childhood. His height continued to grow linearly in adulthood, resembling a eunuch body. The manifestations of metabolic syndrome were abdominal obesity, hyperinsulinemia, non-alcoholic steatohepatitis, impaired glucose tolerance level, increased LDL level, and decreased HDL level. The estradiol level was lower to undetectable and the FSH level increased. Semen examination was basically normal. The patient also presented progressive genu valgum, bone loss, delayed bone age, and unfused epiphysis. The result of *CYP19A1* gene test was c. 1093C>T (p. R365W) homozygous variant, which was a possible pathogenic variant.

In summary, early diagnosis and treatment are the key to aromatase deficiency, and *CYP19A1* gene detection plays an important role in the diagnosis of this disease. The homozygous variant of *CYP19A1* gene c. 1093C>T (p. R365W) is a newly discovered possible pathogenic gene that requires further clinical verification. Clinicians need to raise their awareness of the disease, which can significantly improve the long-term prognosis of patients.

Contributions: LI Hongli Drafted and revised the paper; FU Songbo Proofread and revised the manuscript, and contributed to the analysis of the study; DAI Ruchun, SHENG Zhifeng Contributed to the analysis of the study; LIU Wei Designed the study, proofread and revised the manuscript. All authors have approved the final version of this manuscript.

Conflict of interest: The authors declare that they have no conflicts of interest to disclose.

References

[1] Bulun SE. Aromatase and estrogen receptor α deficiency[J]. Fertil Steril, 2014, 101(2): 323-329. https://doi. org/10.1016/j. fertnstert.2013.12.022.

[2] Bouchoucha N, Samara-Boustani D, Pandey AV, et al. Characterization of a novel *CYP19A1* (aromatase) *R192H* mutation causing virilization of a 46, XX newborn, undervirilization of the 46, XY brother, but no virilization of the mother during pregnancies[J]. Mol Cell Endocrinol, 2014, 390(1/2): 8-17. https://doi.org/10.1016/j.mce.2014.03.008.

[3] Zhu WJ, Cheng T, Zhu H, et al. Aromatase deficiency: a novel compound heterozygous mutation identified in a Chinese girl with severe phenotype and obvious maternal virilization[J]. Mol Cell Endocrinol, 2016, 433: 66-74. https://doi.org/10.1016/j.mce.2016.05.025.

[4] Akçurin S, Türkkahraman D, Kim WY, et al. A novel null mutation in P450 aromatase gene (*CYP19A1*) associated with development of hypoplastic ovaries in humans[J]. J Clin Res Pediatr Endocrinol, 2016, 8(2): 205-210. https://doi. org/10.4274/jcrpe.2761.

[5] Fan LJ, Zhang BB, Li LL, et al. Aromatase deficiency: a case series of 46, XX Chinese children and a systematic review of the literature[J]. Clin Endocrinol, 2020, 93(6): 687-695. https://doi.org/10.1111/cen.14277.

[6] Inas M, Ken M, Aya E, et al. Aromatase deficiency due to a homozygous *CYP19A1* mutation in a 46, XX Egyptian patient with ambiguous genitalia[J]. Sex Dev, 2017, 11(5/6): 275-279. https://doi.org/10.1159/000485278.

[7] Acar S, Erbaş İM, Paketçi A, et al. A novel compound heterozygous variant in *CYP19A1* resulting in aromatase deficiency with normal ovarian tissue[J]. Turk J Pediatr, 2020, 62(5): 826-830. https://doi.org/10.24953/turkjped.2020.05.015.

[8] Costanzo M, Garcia-Feyling J, Saraco N, et al. Accelerated pubertal tempo in a 46, XY aromatase-deficient patient[J]. Horm Res Paediatr, 2018, 90(4): 275-282. https://doi. org/10.1159/000492128.

[9] Dursun F, Ceylaner S. A novel homozygous *CYP19A1* gene mutation: aromatase deficiency mimicking congenital adrenal hyperplasia in an infant without obvious maternal virilisation [J]. Jcrpe, 2019, 11(2): 196-201. https://doi. org/10.4274/jcrpe.galenos.2018.2018.0140.

[10] Shozu M, Akasofu K, Harada T, et al. A new cause of female pseudohermaphroditism: placental aromatase deficiency[J]. J Clin Endocrinol Metab, 1991, 72(3): 560-566. https://doi. org/10.1210/jcem-72-3-560.

[11] Chen ZK, Wang O, Nie M, et al. Aromatase deficiency in a Chinese adult man caused by novel compound heterozygous *CYP19A1* mutations: effects of estrogen replacement therapy on the bone, lipid, liver and glucose metabolism[J]. Mol Cell Endocrinol, 2015, 399: 32-42. https://doi. org/10.1016/j.mce.2014.09.016.

[12] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology[J]. Genet Med, 2015, 17(5): 405-424. https://doi. org/10.1038/gim.2015.30.

[13] Carani C, Qin K, Simoni M, et al. Effect of testosterone and estradiol in a man with aromatase deficiency[J]. N Engl J Med, 1997, 337(2): 91-95. https://doi. org/10.1056/nejm199707103370204.

[14] Saraco N, Nesi-Franca S, Sainz R, et al. An intron 9 *CYP19* gene variant (IVS9+5G>A), present in an aromatase-deficient girl, affects normal splicing and is also present in normal human steroidogenic tissues[J]. Horm Res Paediatr, 2015, 84 (4): 275-282. https://doi.org/10.1159/000437142.

[15] Deroo BJ, Korach KS. Estrogen receptors and human disease [J]. J Clin Invest, 2006, 116(3): 561-570. https://doi. org/10.1172/jci27987.

[16] Mullis PE, Yoshimura N, Kuhlmann B, et al. Aromatase deficiency in a female who is compound heterozygote for two new point mutations in the P450arom gene: impact of estrogens on hypergonadotropic hypogonadism, multicystic ovaries, and bone densitometry in childhood[J]. J Clin Endocrinol Metab, 1997, 82(6): 1739-1745. https://doi. org/10.1210/jcem.82.6.3994.

[17] Lanfranco F, Zirilli L, Baldi M, et al. A novel mutation in the human aromatase gene: insights on the relationship among serum estradiol, longitudinal growth and bone mineral density in an adult man under estrogen replacement treatment[J]. Bone, 2008, 43(3): 628-635. https://doi. org/10.1016/j. bone. 2008. 05.011.

[18] Rochira V, Carani C. Aromatase deficiency in men: a clinical perspective[J]. Nat Rev Endocrinol, 2009, 5(10): 559-568. https://doi.org/10.1038/nrendo.2009.176.

[19] Mukherjee S, Maitra A. Molecular & genetic factors contributing to insulin resistance in polycystic ovary syndrome [J]. Indian J Med Res, 2010, 131: 743-760.

[20] Bremer AA, Miller WL. The serine phosphorylation hypothesis of polycystic ovary syndrome: a unifying mechanism for hyperandrogenemia and insulin resistance[J]. Fertil Steril, 2008, 89(5): 1039-1048. https://doi. org/10.1016/j. fertnstert. 2008.02.091.

[21] Yosipovitch G, DeVore A, Dawn A. Obesity and the skin: skin physiology and skin manifestations of obesity[J]. J Am Acad Dermatol, 2007, 56(6): 901-916. https://doi. org/10.1016/j. jaad.2006.12.004.

[22] Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action[J]. J Clin Endocrinol Metab, 1999, 84(12): 4677-4694. https://doi. org/10.1210/jcem.84.12.6290.

[23] Janner M, Flück CE, Mullis PE. Impact of estrogen replacement throughout childhood on growth, pituitary-gonadal axis and bone in a 46, XX patient with *CYP19A1* deficiency[J]. Horm Res Paediatr, 2012, 78(4): 261-268. https://doi. org/10.1159/000341585.

(Edited by TIAN Pu, CHEN Liwen)

本文引用：李红利, 傅松波, 戴如春, 盛志峰, 刘玮. *CYP19A1* 基因突变致芳香化酶缺乏症 1 例[J]. 中南大学学报(医学版), 2022, 47(6): 794-800. DOI:10.11817/j.issn.1672-7347.2022.210401

Cite this article as: LI Hongli, FU Songbo, DAI Ruchun, SHENG Zhifeng, LIU Wei. Aromatase deficiency caused by mutation of *CYP19A1* gene: A case report[J]. Journal of Central South University. Medical Science, 2022, 47(6): 794-800. DOI:10.11817/j.issn.1672-7347.2022.210401