CASE REPORT

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An extremely rare missense mutation of the androgen receptor gene in a Vietnamese family with complete androgen insensitivity syndrome

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ABSTRACT

We report a Vietnamese family with complete androgen insensitivity syndrome that included several phenotypic females who have a 46,XY karyotype with an extremely rare mutation of the androgen receptor gene. The proband was a 27-year-old phenotypic adult female referred to our department for karyotyping due to primary amenorrhea. Ultrasound examination revealed a small uterus. Chromosomal analysis showed a 46,XY karyotype. A polymerase chain reaction assay revealed the presence of the sex-determining region Y gene. Next-generation sequencing detected the NM_000044.6(*AR*):c.2170C>T(p.Pro274Ser) mutation, which was confirmed by Sanger sequencing. There is only one previous report of this mutation in a child with complete androgen insensitivity syndrome. In the family presented in this study, there were four more phenotypic adult females with primary amenorrhea and a phenotypic female infant with testes in the inguinal canals. The infant (first cousin once removed of the proband) presented with inguinal hernia/swelling in a phenotypic female and one of the four abovementioned adults had similar genetic analysis results. This is the second report of a missense mutation NM 000044.6(*AR*):c.2170C>T in the world and the first study to document a pedigree consisting of several individuals with CAIS as a result of this mutation. The presence of a tiny uterus in the proband, which is a rare occurrence in complete androgen insensitivity syndrome, is a unique clinical indicator of the disorder's variable expressivity.

Keywords: androgen insensitivity syndrome, androgen receptor gene, c.2170C>T (p.Pro274Ser), primary amenorrhea, testes in the inguinal canals

Abbreviations:
CAIS: complete androgen insensitivity syndrome
AR: androgen receptor gene
SRY: sex-determining region Y gene
AMH: anti-Müllerian hormone

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INTRODUCTION

Androgen insensitivity syndrome, OMIM# 300068, was first described as testicular feminization by Morris.[1] It is characterized by resistance to androgen in 46,XY individuals.[2] The external genitalia of patients with androgen insensitivity syndrome vary from a normal female form in complete androgen insensitivity syndrome (CAIS) to an undervirilized male form in partial androgen insensitivity syndrome and a normal male form in mild androgen insensitivity syndrome.[3] The prevalence of CAIS is estimated to range from one in 20,400 to one in 99,100 individuals with a 46,XY karyotype.[2] Primary amenorrhea in a phenotypic female adolescent and inguinal hernia/swelling in a phenotypic female infant are typical scenarios in which CAIS is diagnosed.[4] Although CAIS patients have a female appearance with breast development and complete female external genitalia, they have a 46,XY karyotype with the presence of the *SRY* gene. The clinical presentation of CAIS results from a lack of masculinization caused by ineffective androgen action due to receptor function loss.[5] The androgen receptor is encoded by the androgen receptor (*AR*) gene (OMIM# 313700), which is located on the X chromosome at Xq12.[2] Approximately 95% of CAIS patients have *AR* gene mutation.[6] CAIS is thus an X-linked recessive genetic disorder in which 70% of *AR* mutations are inherited and 30% are de novo.[2] Several hundred *AR* mutations have been identified, and the number is steadily increasing. Since 2012, the Androgen Receptor Gene Mutations Database has added over 800 entries of mutations causing androgen insensitivity syndrome, including over 500 different *AR* mutations.[7] To date, the Human Gene Mutation Database includes more than 700 *AR* mutations, the vast majority of which are missense and nonsense mutations.[8] Thus, it is critical to detect *AR* gene mutations, particularly rare or novel ones in diverse populations, and determine their association with the CAIS phenotype. We present a Vietnamese family with several phenotypic females who have a 46,XY karyotype with an extremely rare *AR* gene mutation.

CASE REPORT

The proband was a 27-year-old phenotypic female adult referred to the Department of Medical Genetics of University of Medicine and Pharmacy, Hue University (Vietnam) for karyotyping because of primary amenorrhea. The physical examination revealed normal female genitalia, sparse pubic and axillary hair, developed breasts, and a height of 158 cm (the average height of Vietnamese women is 153.4 cm). The patient reported no sexual intercourse problems. Biochemistry tests showed serum hormone levels as follows: luteinizing hormone 34.17 mIU/mL, follicle-stimulating hormone 9.13 mIU/mL, estradiol 49.81 pg/mL, testosterone 15.00 ng/mL, and anti-Müllerian hormone (AMH) 23.00 ng/mL. Ultrasound examination showed a small uterus with a longitudinal dimension of 43 mm, an anteroposterior dimension of 12.5 mm, and no endometrium (Fig. 1). No gonad structure was found via ultrasound. A second specialist's ultrasound examination also revealed a small uterus and no gonad structure. Laparoscopy was not available.

Conventional karyotyping of a peripheral blood sample showed a 46,XY karyotype. A polymerase chain reaction assay detected the presence of the sex-determining region Y (*SRY*) gene. Genomic DNA samples was subjected to next-generation sequencing targeting coding region (exome sequencing) using a NextSeq system (Illumina, USA) at Gene Solutions (Ho

Chi Minh City, Vietnam) for a panel of 45 genes associated with disorders of sex development (DSD). The next-generation sequencing results revealed a missense mutation of the *AR* gene, NM_000044.6(*AR*):c.2170C>T p.(Pro724Ser). This substitution was confirmed by Sanger sequencing (Applied Biosystems 3730 Genetic Analyzer; Thermo Fisher Scientific, USA) (Fig. 2). We have submitted this variant to the ClinVar database under the accession SCV001593154.1

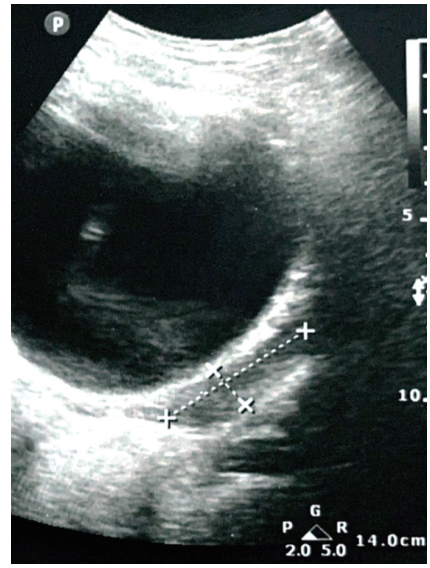

Fig. 1 Ultrasound image of the proband's pelvic area, showing a small uterus with a longitudinal dimension of 43 mm and an anteroposterior dimension of 12.5 mm

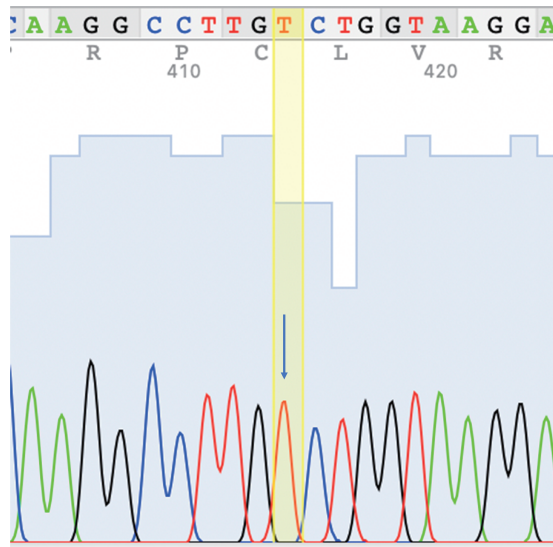

Fig. 2 Sequencing chromatogram of the proband's *AR* gene with NM_000044.6(*AR*):c.2170C>T mutation The arrow indicates the c.2170C>T mutation on the sense strand of the *AR* gene.

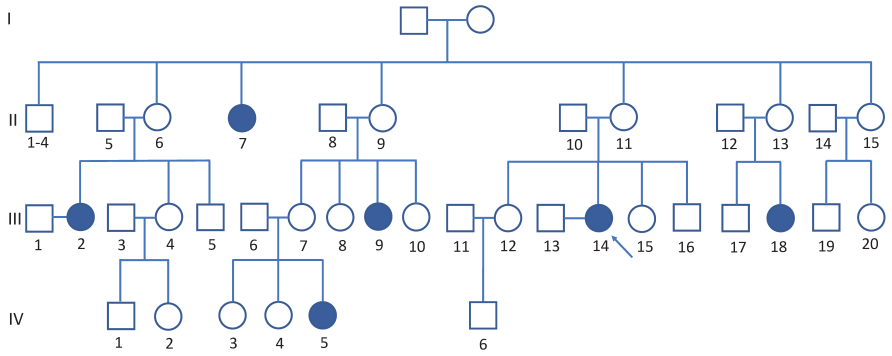

Fig. 3 The pedigree of the proband's family
Members of II-7, III-2, III-9, III-14 (proband), and III-18 were phenotypic adult females with primary amenorrhea; IV-5 was a phenotypic female infant with testes in the inguinal canals.

(VCV001077100.1).

The proband's family included several more phenotypic adult females with primary amenorrhea. They were identified as II-7, III-2, III-9, and III-18 in the pedigree (Fig. 3), with III-9 having palpable masses in the groin bilaterally. Moreover, a phenotypic female infant who was a first cousin once removed (IV-5) of the proband (III-14) had testes in the inguinal canals, suspected due to inguinal swelling and confirmed by ultrasound examination at the age of one month. Karyotyping, polymerase chain reaction assays for the *SRY* gene, and Sanger sequencing for the aforementioned substitution were also performed on III-9 and IV-5. The results showed a 46,XY karyotype, the presence of *SRY* gene, and the NM_000044.6(*AR*):c.2170C>T mutation in both. There was no genetic testing available for other family members with primary amenorrhea.

This research was approved by the Institutional Review Board of the University of Medicine and Pharmacy Hospital, Hue University (approval number 48BV/21).

DISCUSSION

The proband (III-14) in this study was a completely external phenotypic adult female with primary amenorrhea. Karyotyping revealed a 46,XY pattern. Further genetic analysis revealed *SRY* gene positivity. Taken together, these findings led to the diagnosis of a 46,XY DSD. CAIS is the most common disorder that results in a female phenotype in 46,XY individuals.[9] The proband's breast development was consistent with CAIS. While Müllerian structures are not found in most CAIS cases,[2,10] an ultrasound examination revealed that our patient had a small uterus measuring 43 × 12.5 mm. Therefore, Swyer syndrome (complete gonadal dysgenesis) was another possibility in this case. However, the patient's hormonal profile, which included elevated levels of luteinizing hormone, estradiol, testosterone, and AMH, appeared to be more consistent with CAIS than with Swyer syndrome.[11] In general, females with CAIS have normal or higher testosterone levels than males, due to intact testes. Their elevated luteinizing hormone concentrations indicate androgen resistance at the hypothalamic-pituitary level. When testosterone is aromatized, it produces estradiol levels that are higher than those in men but lower than those in women.[3,12] Females with CAIS also have an elevated AMH levels due to the secretion of Sertoli cells.[13] On the other hand, Swyer syndrome has a hormonal profile that includes low testosterone and AMH levels.[11]

Using next-generation sequencing, we detected the patient's *AR* gene variant (NM_000044.6: c.2170C>T), which was located on exon 4 of the *AR* gene. Sanger sequencing confirmed the mutation. The *AR* gene encodes for the androgen receptor protein of 919 amino acids, including three functional domains: N-terminal transactivation domain (residues 1–555), DNA-binding domain (residues 556–623), C-terminal ligand binding domain (residues 666–919), and a hinge region between DNA-binding domain and C-terminal ligand binding domain (residues 624–665).[2,14] The *AR* mutation in this study was a substitution of serine for proline at codon 724 in C-terminal ligand binding domain, which has been recorded on UniProt with an identifier code "VAR_009786" and has been identified as a pathogenic variant in androgen insensitivity syndrome.[15] The proline amino acid is nonpolar and hydrophobic, while serine is polar and hydrophilic. Therefore, this substitution might affect the androgen receptor protein structure and the ligand-binding ability of C-terminal ligand binding domain. The *AR* gene c.2170C>T mutation was also predicted as a damaging variant in bioinformatics tools. It has been reported as a deleterious variant in PROVEAN (score: −6.29; cut-off: −2.5), as a probably damaging effect in Polyphen-2 (score: 0.999; sensitivity: 0.09; specificity: 0.99), and as disease causing in MutationTaster (probability value: 0.999, score: 74, range: 0–215). Furthermore, Hannema[16] reported this mutation as C2529T (P723S) in a 3-month-old child with CAIS as additional evidence of its pathogenic potential. According to the Androgen Receptor Gene Mutation Database, since 2012, there has been a +1 shift in mutation numbering in the DNA-binding domain and the C-terminal ligand-binding domain compared to most previously documented mutations.[7] Consequently, the mutation in our case (Pro724Ser) is identical to the C2529T (P723S) mutation in the Hannema's case.

All evidence considered, our patient was diagnosed with CAIS. Furthermore, the phenotypic female infant (IV-5) with testes in the inguinal canals and one of the females with primary amenorrhea in this family (III-9) had the same genetic analysis results, including a 46,XY karyotype, *SRY* gene positivity, and the *AR* gene c.2170C>T mutation. Unfortunately, IV-5's mother postponed her genetic testing due to a psychological issue. Nonetheless, the fact that IV-5, her maternal aunt (III-9) and her maternal aunt once removed (the proband) had the same CAIS-causing *AR* mutation suggests that the mutation was inherited in the family and IV-5's mother was an obligate carrier of *AR* mutation. It can be speculated that the other three phenotypic adult females with primary amenorrhea (II-7, III-2, and III-18) may also have CAIS due to the same *AR* gene mutation in a 46,XY karyotype. To our knowledge, Hannema was the first to report the c.2170C>T mutation in a child with CAIS. This is the second report of the c.2170C>T mutation and the first study to document a pedigree that includes several individuals with CAIS due to this mutation.

In terms of clinical presentation, most CAIS patients show regression of the Müllerian structures. Surprisingly, our proband still had a small uterus, which was examined by two specialists using ultrasound. The patient did not appear to perceive her vagina to be small or tight and did not report any problems with sexual intercourse. Persistent Müllerian remnants in patients with CAIS were first described by Oka et al.[17] Similar cases have subsequently been reported, albeit very rarely. Some studies have suggested that the presence of Müllerian structures does not exclude a CAIS diagnosis.[18,19] The presence of Müllerian remnants in some CAIS patients demonstrates the variable expressivity of this disorder. The etiology of this phenomenon in CAIS remains unclear. In the first report, Oka et al[17] suggested that it may be attributed to impaired AMH synthesis and/or function. The proband in our study had elevated serum AMH, suggesting that, although Sertoli cells secreted AMH,[13] the hormone could not act on the target organs. Dodge et al[20] also reported a case of CAIS with incomplete Müllerian regression. They hypothesized that the testes descended early, before AMH could affect the Müllerian structures. Both Oka et al's and Dodge et al's explanations might be applicable to our case. We also

performed an NGS assay for the proband with the panel of 45 genes associated with DSD including *AMH* and *AMHR2* genes. No mutations were found in either gene. Further studies are needed to clarify the pathogenesis of this substitution, particularly the association between the *AR* c.2170C>T mutation and the presence of a small uterus, as well as the genetic modifiers that lead to variable CAIS expressivity.

Although the three CAIS patients in this study had the same mutation, their gonad locations were different: inguinal canals in the infant, groins in the III-9, and no location detected using ultrasound in the proband. The risk of gonadal tumors in CAIS patient and the timing of gonadectomy have long been debated. The risk of gonadal malignancy has been estimated to be 14% (0–22%).[21] However, most CAIS patient prefer to postpone gonadectomy due to the risks of the procedure and the subsequent need for hormone replacement therapy. The adult CAIS patient in the current study had not undergone gonadectomy, growing into individuals with a completely female appearance but with no signs of malignancy. Our findings support Hannema's recommendation that gonadectomy be postponed until puberty occurs naturally.[22] Therefore, the infant with CAIS in this study should be advised to keep her gonads until she reaches puberty.

In conclusion, this is the second report of a missense mutation NM 000044.6(AR):c.2170C>T in the world and the first study to document a pedigree consisting of several individuals with CAIS as a result of this mutation. The presence of a tiny uterus in the proband, which is a rare occurrence in CAIS, is a unique clinical indicator of the disorder's variable expressivity.

PATIENT CONSENT

Written informed consent was obtained from the patient's parents for the publication of this case report.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

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