Late diagnosis of complete androgen insensitivity syndrome and transmission/ carriers of the condition in a family with mutation c.2495G> T p.(Arg832Leu) in exon 7 of the androgen receptor gene: genetic, clinical and ethical aspects

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Background. The complete androgen insensitivity syndrome (CAIS) is a rare genetic disorder causing insensitivity to androgens in a person with female phenotype and 46,XY karyotype due to a mutation in the androgen receptor gene located on chromosome X. These children are born with female external genitalia, and females are transmitters.
Case Report. We illustrate an unexpected diagnosis of CAIS in two siblings during examination for short stature, and describe transmission/carriers in the family along with ethical aspects.
Conclusion. A genetic examination could have earlier revealed the transmission of c.2495G>Tp.(Arg832Leu) mutation in exon 7. Our experience highlights the possibility of prenatal testing for the management of pregnancy in a family with a history of CAIS. The implications of prenatal testing in relation to CAIS with clearer explication of ethical and clinical issues warrant further investigation.

Key words: androgen insensitivity syndrome, chromosome X, transmission of the disease, prenatal testing, ethics

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INTRODUCTION

Male development during fetal life only occurs when an XY zygote directs the bipotential gonad to become a testis which secretes sufficient amounts of active androgen to produce the male phenotype[1]. Testosterone (T) stimulates differentiation of the Wolffian duct into epididymis, vas deferens and seminal vesicles. The development of the prostate and masculinization of the primordial external genital into a penis and scrotum requires a more potent androgen, dihydrotestosterone (DHT), which is derived from T via the action of the enzyme 5α-reductase. The mediation of the effect of both T and DHT requires the presence of the androgen receptor (AR) (ref.[2]).

The androgen insensitivity syndrome (AIS; OMIM#300068) is an anomaly of sexual development (DSD) resulting from a complete or partial resistance to the biological actions of androgens in a 46,XY foetus with normal testes determination and normal fetal androgen production. Androgen insensitivity is caused by mutation in the androgen receptor gene, which is located on chromosome X (ref.[3,4]).

Mothers are carriers of this DSD. Patients with a partial androgen insensitivity syndrome (PAIS) have various degrees of virilization of the external genitalia (micropenis, hypospadias, or bifid scrotum, which may contain gonads). Patients with a mild androgen insensitivity syndrome (MAIS) have normal male genitalia and gynecomastia but they are infertile due to azoospermia and oligozoospermia[5].

In complete AIS (CAIS), children are born with phenotypically female external genitalia. Fetal testes in CAIS produce testosterone, but the insensitivity to T and DHT causes defects in the development of the male internal and external genitalia. The production of AMH (anti-Müllerian hormone) in Sertoli cells of the fetal testes is physiological; fallopian tubes, uterus and proximal parts of the vagina from Müllerian structures do not develop. Subjects have a blind-ending shortened vagina. In a systematic review of the literature, the prevalence of CAIS is from 1:20,000 to 99,000 genetic males[5,6].

To advance the field, we report an uncommon case of late diagnosis of transmission of CAIS in a Czech family with an unreported instance of this anomaly. The diagnosis of CAIS in our patient and her sister was entirely unexpected during examination for short stature. In the reported family, this case is associated with missense mutation c.2495G T p.(Arg832Leu) in exon 7 of the an-

drogen receptor gene. To study this further, we aimed to explore the ethical and clinical principles with regard to the possible implications of prenatal testing.

CASE REPORT

Our patient is the first child of non-consanguineous parents. She was born prematuraly in the 36th week of gestation after an uneventful pregnancy by spontaneous delivery. Birth weight and lengtht were 2070 g and 45 cm, respectively. Growth and developing milestones were normal. She had never been seriously ill. The family history was remarkable previously unreported 33-year-old aunt with CAIS who underwent removal of the gonads at the age of 13 years.

Our patient was first seen by an endocrinologist for short stature at the age of 9 years in 2011. Her height was 120 cm (under the 3rd percentile), her BMI (body mass index) was 13,6 kg/m^2 (10th percentile). According to radiological findings, the bone age was 2 years delayed. The growth was below the 3rd percentile without any progressive growth delay. At that time, she was noted to grow in a genetically predicted range related to the parents' heights (the father's height 177 cm, the mother's height 159 cm). She had a proportional stature, female external genitalia without any signs of puberty. On clinical examination, unilateral inquinal hernia was palpable.

Endocrine or chronic disorders as a cause of short stature were excluded. As part of a comprehensive examination, with regard to the family history of CAIS in her aunt, and the suspected inguinal hernia, a cytogenetic examination was indicated, revealing 46,XY karyotype.

The abdominal ultrasound revealed a structure similar to a testis at the interface of the abdomen and the groin on either side. No uterus or ovaries were found. The gynecologist described a blindly ending vagina of 4-5 cm length.

Hormonal follow-up

The test of androgen sensitivity confirmed insensitivity to androgens. During the investigation, there was an absence of decrease in sex hormone binding globulin (SHGB) after administration of depot testosterone 2 mg/kg i.m. (SHGB 95.2 nmol/L prior to the administration of testosterone, SHGB 82.8 nmol/L on the 7th day after the administration of testosterone; the decrease in SHGB was 87%; the required decrease is 82.5%). A sufficient increase in the level of dihydrotestosterone excluded the possibility of 5α-reductase deficiency (dihydrotestosterone 0.11 nmol/L prior to the administration of testosterone, dihydotestosterone 0.87 nmol/L on the 7th day after the administration of testosterone; the increase of dihydrotestosterone was more than twice as much). The level of testosterone was low and the level of gonadotrophin (LH-luteinizing hormone, FSH-follicle-stimulated hormone) was prepubertal. The possibility of 17β-hydroxysteroid dehydrogenase deficiency was excluded.

Genetic analysis

CAIS was confirmed by a molecular genetic examination. Mutation c.2495G T p.(Arg832Leu) in exon 7 of the androgen receptor gene has been detected by Sanger sequencing. The same mutation was confirmed in her 33-year-old aunt with CAIS and her mother and grandmother, who are transmitters of the disease in the family. A subsequent cytogenetic examination of the patient's younger 6-year-old sister also confirmed karyotype 46,XY and the diagnosis of CAIS (Fig. 1). Her hight was under the 3rd percentile too. Both sisters underwent laparoscopic removal of the gonads. The histological examination confirmed atrophic gonads with centrally located fibrosis, tubular structures formed by Sertoli cells, without Leydig cells in the peripheral parts. No germ cell neoplasia was found. Estrogen replacement therapy was started in our patient at the age of 11.

DISCUSSION

CAIS is an uncommon genetic condition that has not been extensively studied in the pediatric literature. Our case study describes two cases associated with familial mutation c.2495G T p.(Arg832Leu) in exon 7 of the androgen receptor gene diagnosed during examination for short stature. We are not aware of any reports associated with CAIS complicated by short stature, however, the underlying pathogenesis of growth retardation is unknown. Of note, the late diagnosis in our part of the family was due to the maternal grandmother of our patient concealing the information from the family about the fact that her daughter (the aunt of our patients) had been diagnosed with CAIS. Therefore, no complex genetic counseling the family which could have revealed the carriers of the disease and suggest the importance of prenatal diagnosis was done.

In CAIS, 46,XY individuals are born with phenotypically female external genitalia. Subjects have a blind-ending shortened vagina. In CAIS, prepubertal patients have T and LH concentrations in the normal range for their age. Testosterone levels are elevated at the time of puberty. Increased LH secretion, due to androgen resistence, stimulates Leydig cell steroid production and results in increased production of testosterone and estradiol with breast development[2]. Patients with CAIS develop breasts as the result of an excess aromatisation of androgens to estrogens, however, have no menses and no pubic or axillary hair or only sparsely. The pubertal growth spurt occurs at the appropriate age[5,6].

In infancy, CAIS may present as an inguinal hernia or labial swelling containing a testis in female infants. Testes can also be located in the pelvis. Bilateral inguinal herniae are rare in female infants, and the incidence of CAIS in such patients is 1-2% (ref.[5,7,8]). In these cases, karyotyping is recommended[5,9]. The vaginal length can be measured in prepubertal girls undergoing inguinal hernia repair. A shortened vagina and the absence of ovaries or fallopian tubes increase the need for karyotyping in these

girls[8]. Alternatively, CAIS can be diagnosed as a result of mismatch between the prenatal sex prediction and the phenotype at birth (ref.[10]), or in the case of a known family history of X-linked CAIS (ref.[5]).

In our case, the diagnosis was made due to the suspected inguinal hernia on the right side and belatedly reported CAIS in a 33-year-old aunt. To date, more than 800 different mutations have been identified in the androgen receptor gene located on chromosome X (ref.[3-5]). Only females can be transmitters of this disease. In our case, missense mutation c.2495G T p.(Arg832Leu) in exon 7 of the androgen receptor gene was found. This mutation was associated with CAIS previously[11]. Furthermore, it was later proven to interfere with the formation of an active AR-ligand complex[12]. The same mutation was also found in the patient′s aunt with CAIS, her mother and her grandmother, who are regarded as transmitters of the disease in the family. Subsequently, cytogenetic examination of the patient′s younger sister also demonstrated the diagnosis of CAIS (Fig. 1).

It is generally known that undescended testes are at a higher risk of undergoing malignant degeneration. In AIS, the risk is low in prepubertal individuals, with an incidence of 0.18%. In adults, the risk estimation of malignancy (seminoma, dysgerminoma) widely varies between 5.5 - 14%, possibly with higher risk in PAIS than in CAIS patients[4,13-15].

Unless gonadectomy is performed in infancy, it is generally recommended to do so in early adulthood in order to avoid the risk of gonadal tumours[5]. A common practice is to remove the testes after puberty, when feminization of the affected individual is complete. Prepubertal gonadectomy is indicated if inguinal testes are physically or aesthetically uncomfortable and if inguinal herniorrhaphy is necessary. After gonadectomy, estrogen replacement therapy is necessary to initiate puberty, maintain feminization and avoid osteoporosis. This replacement therapy must be continuous, however, progesterone is not administered related to the absence of the uterus. The vaginal length may be short and may require dilatation[2]. Our patients underwent bilateral laparoscopic gonadectomy at the age of 9 and 6 years. No germ cell neoplasia was found. We started estrogen replacement therapy in the older sister at the age of 11 years.

Individuals with CAIS are raised as women, and they feel like women, with neurodevelopmental features of female brain in functional studies, however, they are not fertile. Therefore, the important part of the therapy must be psychological support for affected individuals and their families[5,16,17].

Prenatal testing covers many ethical issues including CAIS, however, the public's acceptance of prenatal diagnosis of CAIS has not been well established during the time that testing has been available. Here, the ethical questions are based on the conflict between respecting maternal autonomy versus acting beneficially toward the foetus. Of particular interest are insights into issues of gender identity in subjects with CAIS. In a long-term outcome analysis of CAIS, it was noted that while many patients fare well, dissatisfaction with original gender assignment has been underestimated and gender and sexual counseling should always be made by an multidisciplinary service available to individuals with DSD (ref.[18]). There are at present no answers from our research and previous publications as to the relevance of the ethical challenges[19-21]. Once the pathogenic variant has been identified in an affected family member, prenatal testing for a pregnancy at increased risk and preimplantation genetic diagnosis are possible options. In the case of the foetus having CAIS (ref.[22]), the basic ethical dilema arises whether to undergo pregnancy termination or to keep the pregnancy. Gotlieb et al suggested that requirements for prenatal testing for conditions which (like CAIS) do not affect the intellect and have treatment available are not common. Nevertheless, differences in perspective exist among medical professionals and in families regarding the use of prenatal testing, particularly if the testing is being considered for the purpose of pregnancy termination rather than early diagnosis. Most centers would consider decisions about prenatal testing to be the choice of the parents[23]. Related to this point the decision whether to keep the pregnancy based on a positive result is usually a difficult one involving legal, psychosocial, political and cultural considerations and religious beliefs. But we do urge caution here. Caregivers in the interest of facilitating appropriate medical care will have an important role in helping the couples elicit their own preferences about testing to make informed reproductive choices. We recommend that further research aims to confirm the importance of psychosocial and ethical factors.

CONCLUSION

From a practical point of view, two important points are raised by this case. Firstly, the diagnosis of CAIS in our subjects was unexpected during the examination for short stature.

Secondly, CAIS and its transmission in the family were correctly diagnosed. Our findings support the assumption that a complex genetic examination could have revealed the transmission of missense mutation in the androgen receptor gene in this family earlier highlighting the importance of prenatal testing to help the couples make informed reproductive choices in families with history of AIS. We reaffirm the need for reflecting the ethical and psychosocial implications of introducing prenatal diagnostic tests and further research in this area, should help to build a more comprehensive understanding of personal preferences, the emotional, behavioral and personality factors in CAIS.

ABBREVIATIONS

AIS: androgen insensitivity syndrome; CAIS: complete androgen insensitivity syndrome; MAIS: mild androgen insensitivity syndrome, T: testosterone; DHT: dihydrotestosterone; AR: androgene receptor; DSD: disorders of sex development; AMH: anti-Müllerian hormone; SHGB:

sex hormone binding globulin; LH: luteinizing hormone, FSH: follicle-stimulated hormone.

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