AACE Clinical Case Rep. 7 (2021) 216–219

Contents lists available at ScienceDirect

AACE Clinical Case Reports

journal homepage: www.aaceclinicalcasereports.com

Case Report

A Family With Novel X-Linked Recessive Homozygous Mutation in *ANOS1* (c.628_629 del, p.1210fs*) in Kallmann Syndrome Associated Unilateral Ptosis: Case Report and Literature Review

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ARTICLE INFO

Article history:
Received 25 December 2020
Received in revised form
14 January 2021
Accepted 19 January 2021
Available online 17 February 2021

Key words:
ANOS-1
anosmia
congenital ptosis
hypogonadotropic hypogonadism
Kallmann syndrome

ABSTRACT

Objective: Kallmann syndrome (KS) may be accompanied by anosmia or hyposmia and midline defects. We present an overweight 16-year-old boy with a lack of puberty, anosmia, congenital right eye ptosis, and normal intellectual function.
Methods: Testicular ultrasonography was performed. Whole-exome sequencing was performed on peripheral blood specimens. Genetic results were confirmed by Sanger sequencing. Anosmia was evaluated quantitatively using the Korean version of the Sniffin' stick test II.
Results: Our patient presented with a complaint of lack of body hair growth and small penile size with no remarkable medical history. He was the second son of third-degree consanguineous healthy parents. Physical examination revealed pubertal Tanner stage I. Congenital right eye ptosis and obesity were noted. Anosmia was confirmed. The laboratory evaluation revealed a low serum level of testosterone, follicle-stimulating hormone, and luteinizing hormone. An X-linked recessive homozygous mutation, c.628_629 del (p.1210fs*) in exon 5 of the *ANOS1* gene was revealed and was also found in the patient's uncle and great uncle on the mother's side.
Conclusion: To date, approximately 28 *ANOS1* mutations producing KS phenotypes have been described. However, to the best of our knowledge, this particular X-linked recessive mutation has not been previously reported in KS. Furthermore, ptosis is a rare finding in KS literature. Identification of these cases increases awareness of the phenotypic heterogeneity in novel forms of KS, thereby expediting early definitive treatment, which may prevent the development of further complications.

Introduction

Congenital hypogonadotropic hypogonadism (HH) is a genetic disease that prevents typical pubertal development in both genders and is also responsible for infertility.[1] It is accompanied by either normal olfaction or altered odor perception, the latter known as Kallmann syndrome (KS).

Inadequate amounts of follicle-stimulating hormone, luteinizing hormone, testosterone, estradiol, and progesterone are the unfavorable results of gonadotropin-releasing hormone (GnRH)-secreting cell migration failure, leading to impaired secondary sexual characteristics, including failure of menstruation in women and underdeveloped testes in men.[2] Furthermore, there is a direct relationship between manifestation and the level of sex hormones.[3]

KS is the result of sporadic or familial genetic imbalance. Familial KS can be inherited in Mendelian autosomal recessive, autosomal dominant, or X-linked patterns.[4] Considerable amounts of monogenic defects are associated with KS, including mutations in *FGFR1*, *PROK2*, *PROKR2*, and most of all, *ANOS1*, which was previously named *KAL1*.[5] *ANOS1* is located in the Xp22.3 region[6] and

Abbreviations: GnRH, gonadotropin-releasing hormone; KS, Kallmann syndrome.

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https://doi.org/10.1016/j.aace.2021.01.007

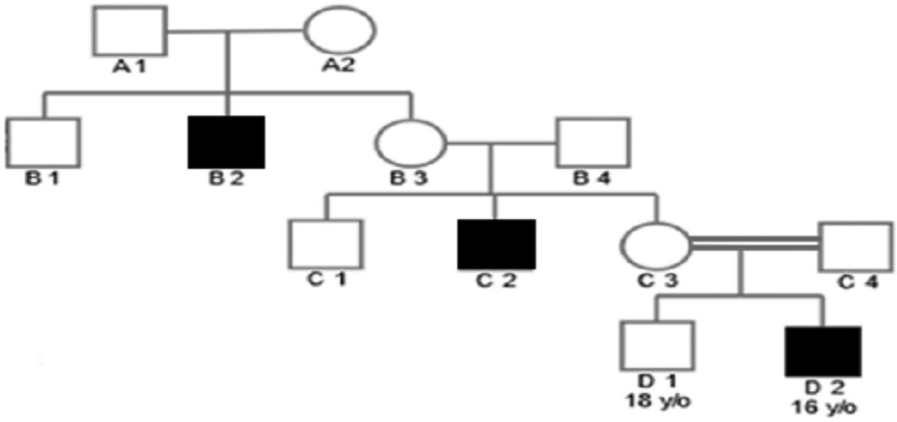

Fig. 1. Pedigree of the index family. Females are represented as circles, males as rectangles, and confirmed cases of Kallmann syndrome as solid symbols. Genetic analysis was first performed on D-2 and C-3, which revealed an X-linked recessive homozygous mutation in D-2 and heterozygous mutation in C-3. Due to the X-linked inheritance of the *ANOS1* gene, we did not perform any analysis on C-4. Furthermore, an analysis of C-2 and B-2 revealed the same mutation. As stated previously, the father (C-4) is the mother's uncle's grandson; hence, B-2 is the grandfather of the proband (D-2).

encodes anosmin, which is responsible for the migration of GnRH-producing neurons.[7]

Early diagnosis of sporadic KS remains challenging, mostly due to the lack of similar cases in the family, the protean manifestations of the disease, and limited evidence of *ANOS1* coding region mutations. Here, we present an Iranian boy presenting with a lack of puberty and 2 members of his family, his uncle and great uncle on the mother's side, carrying an X-linked recessive homozygous novel mutation of the *ANOS1* gene. We also provide a review of the literature.

Case Report

A 16-year-old Iranian male (D-2 in Fig. 1) presented to the Endocrine Unit of our pediatric hospital with complaints of lack of body hair growth, small penile size, and absence of voice deepening. He was the second child of the family born to healthy consanguineous (third-degree) parents. The father is the mother's uncle's grandson.

On examination, he looked younger than his chronological age. Pubertal Tanner stage I (G1, P1) was revealed. He had no beard or pubic hair. Following height (172 cm) and weight (88 kg) measurements, he was categorized as overweight grade 1, bordering on grade 2, due to a body mass index of 29.7, based on the interpretation of anthropometric indices of the World Health Organization. No signs of midline defects, including a scar on the palate or lip or congenital cleft lip or palate, were observed. On examination, the patient demonstrated no sign of gynecomastia. In the dental examination, all permanent teeth except 4 third molars were developed, and there was no sign of impacted teeth. The Adam test found no evidence supporting scoliosis. He also suffered from right eye ptosis since birth.

The patient was born following an uneventful full-term pregnancy. At birth, he weighed 3.7 kg, measured 53 cm in crown-heel length, and had a head circumference of 37 cm. School performance and intellectual function were typical. No neurologic concerns except ptosis and anosmia were revealed. No documented abnormal clinical and paraclinical evidence supporting renal abnormalities (including agenesis) existed.

There was no medical history of cardiopulmonary involvement and related associations (eg, CHARGE syndrome: coloboma, heart anomalies, atresia of the choanae, retardation of growth and development, and genital and ear anomalies). Anosmia confirmation was performed quantitatively using the Korean version of the Sniffin' stick test II. His stretched penile length was 36 mm, and testes were palpated on both sides. History of undescended testicles was neither mentioned nor documented.

The sexual hormone profile results were as follows: follicle-stimulating hormone, 0.16 IU/L(normal age-gender adjusted, 0.3-10 IU/L); luteinizing hormone, 0.86 IU/L (normal age-gender adjusted, 1.5-8.0 IU/L); and testosterone, 135 ng/dL (normal, >300 ng/dL). Baseline T4 and thyrotropin were within in the normal range. The adrenal hormone level was average.

Concerning the pedigree depicted in Figure 1, C-2 had an ambiguous history of micropenis in childhood, and B-2 had documented unilateral ptosis. The family history was otherwise unremarkable. With the impression of HH, genetic analysis was performed using whole-exome sequencing on peripheral blood samples. A variant was identified in the *ANOS1* gene: c.628_629 del AT in exon 5 (p.1210fs*). This mutation was neither found in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) nor the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and was classified as a pathogenic variant based on the VarSome database (https://varsome.com/).

Whole-exome sequencing for C-2 and B-2 (Fig. 1 and 2) revealed the same mutation. Further genetic analysis demonstrated that C-3 (D-2's mother) was a heterozygous carrier of this gene. All genetic findings were confirmed by Sanger sequencing. The Sanger sequencing results of D-2 and C-3 are presented in Figure 2.

To the best of our knowledge, it is the first case of KS with this variant of *ANOS1*. The patient is currently on a testosterone treatment regimen and in the third month of monthly follow-up visits. We are further evaluating his condition.

Informed consent was obtained from the parents of the patient for inclusion in this study.

Discussion

KS is a rare condition with an estimated incidence of more than 1 in 48 000 individuals.[8] KS can present as hyposmia or anosmia. Although the underlying etiology of KS is not well-known, altered odor perception and gonadal function are believed to be due to the disturbed migration of olfactory neurons and GnRH-secreting cells from the olfactory placode region to the hypothalamus.[9]

We reported an Iranian family diagnosed with KS carrying a novel mutation in the *ANOS1* gene. The 16-year-old male patient (D-2 in Fig. 1) presented with delayed puberty, hypogonadism, anosmia, and ptosis. The patient harbored a 2-base frameshift

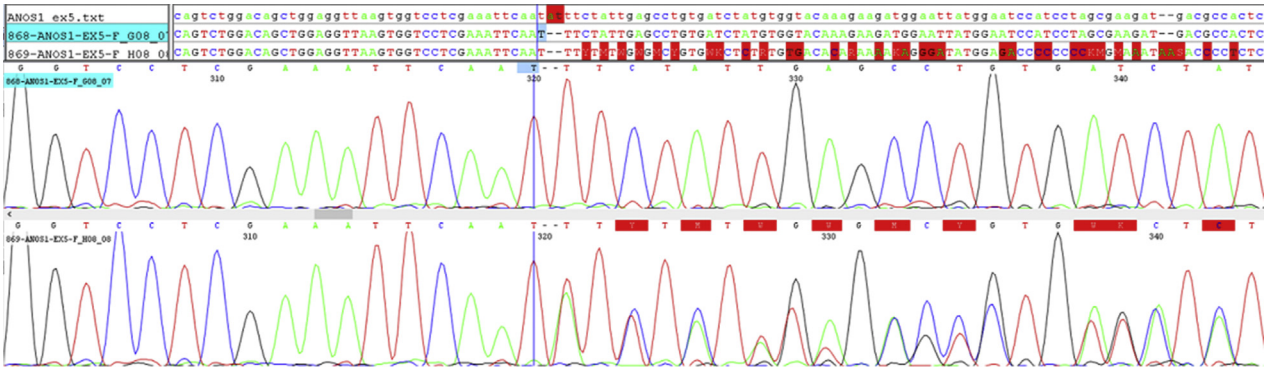

Fig. 2. Sanger sequencing results of the main presenting patient (D-2) in the upper row and his mother in the lower row. The mutation is located at codon 320.

deletion, leading to a p.1210fs* mutation. Although not every patient with hypogonadotropism and anosmia is diagnosed as KS,[10] it remains the most probable differential diagnosis for the mentioned symptoms. Male patients suffering from HH require testosterone, and patients should be closely monitored during treatment.[11]

Isolated deficiency of gonadotrophin or KS was first identified and reported by Kallmann et al in 1944.[12] Results of previous studies strongly suggest that no mutation involving the *ANOS1* coding region has been identified in families with only males affected.[13] A considerable number of patients in the literature were diagnosed in adolescence and young adulthood when they presented with arrested or absent puberty.[14] Likewise, the presented case in this article was admitted in the second decade of life, complaining of delayed puberty.

It is noteworthy that sporadic cases of KS seem to be much more frequent than familial cases.[15] Sporadic cases account for nearly 60% of all patients.[13] Previous studies reported that the incidence of defects in the *ANOS1* encoding region in sporadic cases does not exceed 8%.[16] Gender distribution in male patients is 5- to 6-fold more than in females (1:10 000 compared with 1:50 000), suggesting predominance of X-linked inheritance compared with other patterns.[17] Furthermore, KS has proved to be more common in the Maghrebian people than Europeans.[18]

To the best of our knowledge, based on the VarSome database, 22 genes have been recognized as correlated with KS. One of the most crucial of these is *ANOS1*, previously named *KAL1*, which contains 56 pathogenic and 11 likely pathogenic variations of the *ANOS1* gene leading to various diseases. Our searches of other databases, including ClinVar and the Human Gene Mutation Database, did not find a pathogenic report of c.628_629 del in any diseases. In other words, based on the ClinVar database, there are 28 KS-related pathogenic variants of the *ANOS1* gene, and the c. 628-629 deletion was not one of them.

Other genes correlated with the KS phenotype, including *CCDC141*, *FGF8*, *FGF17*, *PROKR2*, *CHD7*, and *DUSP6*, among others, have been reviewed in other papers.[4,19-25] Raivio et al suggested that 7.8% of patients with a combined pituitary hormone deficiency had mutations in at least one of *FGFR1*, *FGF8*, or *PROKR2* genes.[23] Moreover, in 2016, Hutchins[19] postulated that *CCDC141* plays a crucial role in the embryonic migration of GnRH-containing neurons and eventually initiates pulsatile GnRH secretion.

Two members of this family (D-2 and B-2) presented with unilateral eye ptosis since birth. Although rare, congenital ptosis as the first presentation of KS has been reported in other patients. In 2007, Reardon et al presented 2 siblings diagnosed with KS with congenital ptosis who also had a frameshift mutation in the *ANOS1* locus.[25] Ocular involvements in KS may also include oculomotor abnormalities, coloboma, congenital fibrosis of the extraocular muscle, optic atrophy, and color blindness.[25,26] Some studies have attributed part of these eye abnormalities to the midline facial defect, which is also responsible for other nonreproductive features of KS.[27]

Although further studies are required to define genotype-phenotype correlation, it is recommended to consider KS in patients suffering from hypogonadism and eye disorders. Furthermore, in KS cases, we highly recommend analyzing other members of the family to discover any potential mutations. Accurate history taking and comprehensive clinical evaluation are key in diagnosing such rare disorders.

Acknowledgment

We thank all those who were involved in this study.

Disclosure

The authors have no multiplicity of interest to disclose.

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