

Case report

Genetic diagnosis of idiopathic hypogonadotrophic hypogonadism: a new point mutation in the KAL2 gene

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ABSTRACT

Kallmann Syndrome (KS) is a genetic disease of embryonic development which is characterized by the association of hypogonadotropic hypogonadism (HH) due to a deficit of the gonadotropin-releasing hormone (GnRH) and a hypo/anosmia (including a hypoplasia of the nasal sulcus and agenesis of the olfactory bulbs). Even though it is a genotypically and phenotypically heterogeneous clinical disease, there are some key genes related to KS (KAL1, FGFR1 (KAL2), GNRHR, KISSR1 (GPR54), GNRH1, NELF and PROK2). The aim of this study was to present a case report of a genetic diagnosis of KS linked to the presence of mutations in the FGFR1 (fibroblast growth factor receptor 1, also known as KAL2) gene. This diagnosis was made in a 44-year old female affected by a hypogonadism for which she had received intermittent treatment until she was 30 years old based on the patient's own decision. The molecular analysis of FGFR1 identified the mutation c. 246_247delAG (p.T82Xfs110) in heterozygosis on exon 3 of the KAL2 gene. This is the first report of this mutation related to idiopathic hypogonadotropic hypogonadism (IHH).

Key words: Case, Genes, KAL1, KAL2, Kallmann syndrome, Mutation

INTRODUCTION

Kallmann Syndrome (KS) is the most frequent cause of congenital hypogonadism with an incidence of around 0.1 and 0.2%. It is characterized by the as-

sociation of optic atrophy, deafness, a cleft lip, renal malformations, cryptorchidism and neurological anomalies.^{1,2} This disorder is a form of hypogonadotropic hypogonadism (HH), which is a condition affecting the production of hormones that facilitate sexual development. Males with hypogonadotropic hypogonadism are often born with an unusually small penis (micropenis) and undescended testes (cryptorchidism). They also present with delayed or incomplete puberty. Affected females usually present with primary amenorrhea and do not have a completed secondary

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sexual development, such as having little or no breast development.^{3,4} KS and other syndromes causing a congenital deficiency of gonadotropin-releasing hormone (GnRH) are characterized by low levels of LH and FSH with low levels of sexual steroids (testosterone and estradiol).⁵ In some sporadic cases of hypogonadotropic hypogonadism, aetiologies that may disrupt the communication pathway between the hypothalamus and pituitary should be excluded.^{3,5,6} KS is genetically heterogeneous and the majority of cases (around 60%) are presented as sporadic cases (only one person affected in the family).⁴ In familial KS, autosomal recessive, autosomal dominant and X-chromosomal recessive inheritances have been described.⁹ The principal genes involved in the aetiology of KS are KAL1,¹⁰ FGFR1,¹¹ FGF8,¹² PROK2,⁷ PROKR2⁷ and WDR11.^{4,13}

Defects in the KAL1 gene, localized on chromosome Xp 22.3, are responsible for preventing embryonic migration of olfactory nerve cells. In at least one genetic form of the disease, deficiency in human gonadotropin results from impeding the migration of GnRH neurons, from the olfactory placode to the hypothalamus, during embryonic life.¹⁴ If olfactory nerve cells do not extend to the olfactory bulb, a person's sense of smell will be impaired or absent.¹⁵ Apart from mutations in the KAL1 gene, there are other genetic anomalies that cause isolated deficiencies of GnRH, including recessive and dominant autosomic alterations. The FGFR1, PROKR2, GnRHR, GnRH, TAC3, TACR3, NELF and PROK2 genes also play a role in the migration of neurons that produce GnRH.¹⁶ GnRH controls the production of several other hormones that guide sexual development before birth and during puberty. These hormones are important for the normal function of the gonads.^{4,17}

Due to suspicion of KS, a genetic analysis in a 44-year old woman was requested with the chief objective of identifying the main mutations in genes KAL1, FGFR1 (KAL2), GnRHR, KISSR1 (GPR54), GnRH1, NELF and PROK2. Genetic data and clinical information confirmed a case of IHH and included new mutations for this pathology.

CASE REPORT

A 44-year old woman was referred to the endo-

crinology unit for hypothyroidism, which had been diagnosed 3 months previously. Her investigations showed: TSH (thyroid-stimulating hormone) values 59.96 Uu/ml (NR: 0.5-4.25 uUI/ml) and T4 F (free thyroxin 4) values 0.41 ng/dl (NR: 0.54-1.9 ng/dl). There is no information about the presence of goiter. Anamnesis produced information about other symptoms such as tiredness (which could not, however, be quantified), skin dryness, dysphonia, oedema, constipation, and also showed that she had had her primary amenorrhoea controlled by the Gynaecologic Service since adolescence. However, the patient did not present an alteration in olfactory capacity.

At the age of 30 years she wanted to conceive and attended a reproduction service to be treated by stimulation and in vitro fertilization-embryo transfer (IVF-ET). In the preconception study the vaginal smear showed hypoestrogenism and in TAC the hypophysis yielded a normal result. For these reasons, the patient continued treatment with agonists of GnRH and gave birth to twins: a male with a cleft lip and palate which needed to be surgically treated, and a healthy female. After labor the patient discontinued the hormonal treatment and continued with the analysis of primary hypothyroidism.

According to the patient's gynaecology medical history, she was first studied at 15 years old. Genetic analysis determined her karyotype as one showing normal characteristics. The gynaecologic ecography image showed a uterus at an abdominal-pelvic level. With these analytical results a diagnosis of hypogonadism was confirmed, so the patient intermittently continued treatment with different drugs controlled by continued analysis until she decided to cease all pharmacological treatments at the age of 22 years old.

Family members' clinical information

There are some family precedents. For example, her mother developed breast cancer, her brother had cerebral palsy but without symptoms of sexual development, and her sister had primary amenorrhoea.

Physical Examination

Weight: 70.3kg; height: 170.5 cm; mean arterial pressure (MAP): 115/65 mmHg. No goiter. Female normal phenotype with breasts at Tanner IV stage and presence of a few pilose follicles in armpit and pubis

(Tanner II). The gynaecologic exploration showed the presence of small clitoris and labia minora.

Additional Tests

- TSH 52.96 uUI/ml (NR: 0.4-4 mU/L), T4F 0.41 ng/dl (NR: 0.2-8 ng/dl), positive TPO, prolactin 9.1 ng/dl (NR: 3.8-23.2 µg/l), FSH 9.05 UI/L (NR: 3-20 UI/L, values depending in the ovulating period), LH 1.52 UI/L (NR: 8-20 UI/L), E2 37 pg/ml (NR: 30-300 ng/L), testosterone 0.6 ng/dl (NR: ≥ 0.6 µg/L), Cortisol 14.4 µg/dl (NR: 30-250 mg/L), HGH 0.1 ng/mL (NR: 1 - 16 ng/mL) and somatomedin C 92 ng/ml (NR: 101 - 267 ng/ml).
- LHRH test LH 2.4-14.8-18 and FSH 7.3-16.6-21.4 UI/L.
- Densitometry: z-score of -2.3 in lumbar area and -1.9 in cortical bone.

For the genetic analysis of this patient, DNA from blood was extracted by QIAamp DNA Blood Mini Kit (Qiagen)[®]. After the amplification process of all the 18 codifying exons and adjacent introns area in gene FGFR1 (KAL2, NG_007729.1), and the sequencing process, the samples were visualized in a sequencer 3130 Genetic Analyzer (Applied Biosystems). The obtained sequence was compared to the consensus sequences for the FGFR1 gene (GenBank Accession Number: NM_023110.2). The same procedure was followed in the KAL1 gene by amplifying and sequencing the 14 exons and the adjacent introns of this gene. Firstly, we performed a genetic analysis of the main genes related to Kallmann syndrome (KAL1, FGFR1 (KAL2), GNRHR, KISSR1 (GPR54), GNRH1, NELF and PROK2)^{11,19} using the Multiplex Ligation Probe Amplification (MLPA) technique, covering the large deletions and duplication areas. After this first analysis in these main genes, we performed a detailed analysis in genes KAL1 and KAL2 searching for specific point mutations.

Genetic analysis

The molecular analysis of the fibroblast growth factor receptor 1 (FGFR1), by sequence analysis of all codifying regions (exons 1-18), including the adjacent intronic regions, allowed the identification of the mutation c. 246_247delAG (p.T82Xfs110) on exon 3 in heterozygosity (Figure 1). Two other heterozygous variations were found: c.1600 G>R

(p.V534I) at exon 11 and c.1833 C>Y (p.I611I) at exon 12¹⁸ of the KAL1 gene.

DISCUSSION

The existence of altered thyroid function combined hypogonadotropic hypogonadism and other clinical characteristics like delayed puberty, infertility and primary amenorrhea among others, allowed the clinical diagnosis of KS.^{6,20} The molecular identification of a new mutation on the FGFR1 gene and two mutations in the KAL1 gene corroborated it.

The FGFR1 (KAL2) gene (8p11.2-p12) consists of 18 exons and encodes one member of the FGFR family, FGFR1. FGFR1 consists of three extracellular Ig-like domains D1-D3, an acid box domain, one transmembrane domain and one tyrosine kinase domain.¹⁵ Heterozygous deletions, non-sense mutations and missense mutations in the FGFR1 gene have been shown to underlie an autosomic dominant form of KS.^{7,11} This case expands our knowledge of the FGFR1 mutations causing KS by identifying a novel mutation, c. 246_247delAG. Due to the genetic characteristics of Kallmann syndrome, which have been demonstrated to be autosomal dominant type, it is quite possible that the clinical features of this particular case could be the result of the mutation found. More specifically, this mutation most probably causes a frameshift change in mRNA which originates a stop point at codon 110. Eventually, it causes a truncating protein with a loss of 95.53% of the amino acids and generates an altered structure and function of the normal protein product. Although the mutation identified in this case has not yet been published in previous literature or added to the Human Gene Mutation Database Professional, it can be regarded as pathogenic since protein function is compromised, this being consistent with the diagnosis of KS (OMIM #147950). There are many other mutations in the FGFR1 gene, some of them related to this syndrome, like c.286_288delTCC deletion in KS²¹ and other mutations like R254W and R254Q in IHH.²²

The KAL1 gene (Xp22.3) is one of the other main genes that cause KS with an X chromosome-linked form. It encodes anosmin-1, an extra-cellular glycoprotein, to around 95 kDa of unknown function. Anosmin-1 has a compound modular structure,

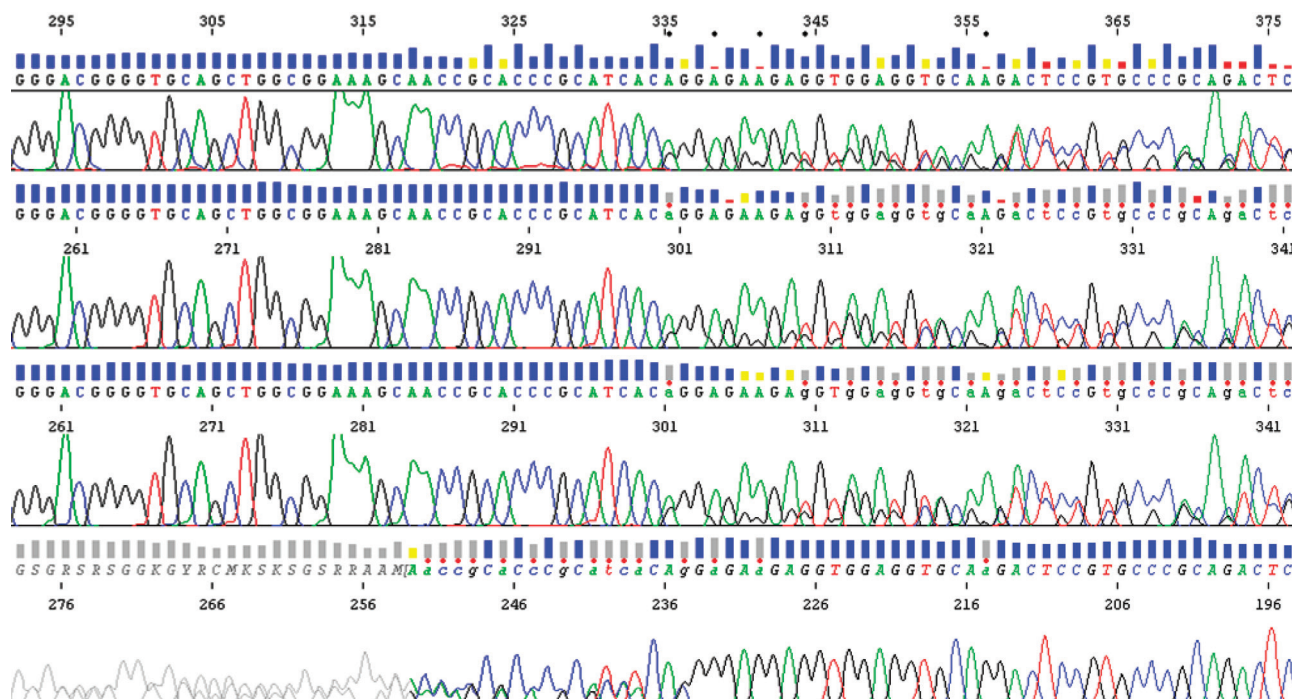


Figure 1. Sequencing of the KAL2 gene. Electropherogram overlapping the novel mutation. Mutations are indicated by a vertical arrow on the electropherogram. Mutation causes the loss of two base pairs in heterozygosis.

comprised of a large cysteine-rich amino-terminal region including a whey acidic protein (WAP)-like four-disulfide core motif, 4 fibronectin-like type III (FnIII) repeat and a short carboxyterminal region rich in basic amino-acids. Anosmin-1 binds to heparan sulfate glycosaminoglycans, but the precise mechanism by which anosmin-1 and FGFR1 cooperate in FGF-signalling is still unknown.²³ We reported this new point mutation in the KAL2 gene which, in combination with the mutations in the KAL1 gene (p.V534I and p.I611I), seem to be important factors for development of KS. Therefore, the various KAL1 and FGFR1 mutations underlying KS are believed to be loss-of-function mutations, and KS is likely to result from insufficient FGFR1-mediated signalling during embryonic development.^{11,23} However, only around 20% of the patients^{19,23} affected by KS display a mutation either in KAL1 or FGFR1, indicating that there are other as yet undiscovered KS genes.²³ Furthermore, new mutations in both these genes are continuously being discovered, as in the present case.

In conclusion, the present case report provides evidence for the important role of genetic testing in

clinical practice which appears to be crucial due to the identification of an increasing number of mutations that disturb the development and function of the hypothalamic-pituitary-gonadal (HPG) axis and cause disturbances in pubertal development. Most mutations have effects at all levels of the HPG axis and are inactivating, thus causing hypogonadism and arrest or delay of pubertal development.²⁴ The correct diagnosis of these disorders using molecular biological techniques is now possible. This allows for the selection of specific treatments and correct counselling of the patients and their families. Due to increasing insights into complex genetic diagnosis in idiopathic GnRH deficiency (IGD), the identification of new genes and mutations is allowing the profession to classify IGD more precisely and provide more complete information to patients about their diagnosis and the recurrence risk for their family members.²⁵ Thus, these advances will lead to reduction of the cost of undertaking large screening in genes by sequencing wide areas and genetic identification will be more accurate. Additionally, physicians will be increasingly enabled to explain complex genetic concepts to patients who go to the clinic as well as to make clear

what they should expect, both in regard to symptoms and the likelihood that their children, siblings or other family members may also be affected.²⁵

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The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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