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ASSISTED REPRODUCTION TECHNOLOGIES

Live birth in male de novo Kallmann syndrome after cross-generational genetic sequencing

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Abstract

Purpose To present the first case proposing the use of preimplantation genetic testing for monogeneic disorders for Kallmann syndrome, providing comprehensive care in the genomic era of precision medicine.

Methods Gonadotropin therapy was used for spermatogenesis, followed by in vitro fertilization by intracytoplasmic sperm injection and embryo transfer. Cross-generational targeted next-generation sequencing was then done for genes known to cause Kallmann syndrome.

Results A heterozygous mutation at codon 102 of the FGFR1 gene was found in the patient, but the father was found to have the same mutation yet is unaffected by Kallmann syndrome. Since no causative mutation was found, a de novo or sporadic mutation was suspected as the cause of Kallmann syndrome in this case.

Conclusions Comprehensive care must be available for male Kallmann syndrome patients, as treatment should not stop at spermatogenesis, but continue with genetic counseling due to possible inheritance.

Keywords Kallmann syndrome · Next-generation sequencing · Genetic counseling · Spermatogenesis

Introduction

Kallmann syndrome (KS) is characterized by hypogonadotropic hypogonadism and olfactory abnormalities [1]. KS prevents normal pubertal development and causes infertility in patients. It is caused by impairment of olfactory axon development and failure of migration of gonadotropin-releasing hormone (GnRH) neurons [2, 3]. It is found more commonly in males than females, with an incidence rate of 1/4,000–1/10,000 in males [1]. There are currently over thirty known gene mutations that are associated with KS and that account for 50% of known cases [4, 5]. Though infertile at presentation, most KS patients have a good reproductive prognosis [4, 6], and therefore require adequate genetic counseling. Here, we present a case where a male KS patient opted for preimplantation genetic testing after using gonadotropin therapy for spermatogenesis, in addition to in vitro fertilization by intracytoplasmic sperm injection and embryo transfer.

Case

This 35-year-old married male patient presented at our infertility clinic. He reported being diagnosed with Kallmann syndrome at age 21 during a routine health exam, after the physician found anosmia and lack of secondary sexual characteristics. He underwent continuous testosterone replacement therapy since then and at the age of 33 years, after 2 years of marriage, he was found to have azoospermia. Members of his immediate family (his father, mother, and one brother) had normal pubertal development and no anosmia.

At his first infertility clinic visit, physical exam revealed body height of 177 cm and body weight 94 kg, and normal-sized penis and testicles. The patient also reported normal erection and ejaculation function. His lab data showed FSH 1.46 mIU/mL, LH 0.44 mIU/mL, and testosterone 1.47 ng/ml. He discontinued his regular testosterone medication and then began weekly human chorionic gonadotropin (hCG) 1500 IU injections and human menopausal gonadotropin (HMG) 150 IU injections three times a week. The hormone levels over the course of treatment are shown in Table 1. Five months

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Table 1 Patient's hormone profile and semen analysis during gonadotropin therapy

Month after beginning treatment	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)	Semen analysis		
				Concentration (per mL)	Motility	Morphology
0	1.46	0.44	1.47	(Not tested)		
1	7.76	3.46	2.07	(Not tested)		
2	6.45	3.29	1.23	(Not tested)		
3	6.52	2.18	3.42	(Not tested)		
4	7.10	3.54	0.97	(Not tested)		
5	5.99	2.81	2.26	0.1×10^6	67%	65%
6	7.11	1.72	4.18	1×10^6	100%	50%
7	5.02	1.77	3.83	0.2×10^6	20%	50%
8	5.57	2.40	2.10	0.01×10^6	30%	30%

after beginning treatment, sperm was seen during semen analysis at 0.1×10^6/mL. Due to low sperm count, it was recommended the couple undergo in vitro fertilization with intracytoplasmic sperm injection with fresh sperm.

The patient's wife was 32 years old, and had a history of regular menses, and her AMH was 4.05 ng/mL. She underwent controlled ovarian stimulation when sperm was confirmed to be found in semen analysis. Due to the couple's concern about the inheritance of Kallmann syndrome and after thorough discussion, preimplantation genetic testing for both aneuploidy (PGT-A) and monogeneic disorders (PGT-M) was planned to be done. Twelve oocytes were retrieved and fertilized with fresh sperm. After 5 days of culturing using Life Global medium, 3 blastocysts (Gardner grading 2AA, 1BB, 1BB) [7], 3 early blastocysts, 1 morula, and 1 nine-cell embryo (cleavage stage grade 3) [8] were vitrified using the Cryotop device and commercially available vitrification solutions (Kitazato BioPharma Co., Shizuoka, Japan). Concurrently, the patient received targeted next-generation sequencing genetic analysis focusing on over thirty genes known to cause Kallmann syndrome, as found on the NCBI Genetic Testing Registry panel gene list at that time. Sequencing was performed using the Illumina NextSeq 500 platform with Agilent SureSelect Library (Santa Clara, CA USA) with average coverage of ≥ 20× for 99.99% of the targeted genes, along with Burrows-Wheeler Aligner (BWA), Genome Analysis Toolkit (GATK) algorithm, and the Ensembl Variant Effect Predictor. Variants with a minor allele frequency (MAF) < 0.01% in 1000 Genomes Project were identified and confirmed with Sanger sequencing. He was found to have a heterozygous variation at codon 102 of the fibroblast growth factor receptor 1 (FGFR1) gene, causing an amino acid change from valine to isoleucine (Fig. 1). However, when the patient's mother and father were tested as well, the father was found to have the same variation but was unaffected by Kallmann syndrome. This variation is listed in the Human Genome Mutation Database [9] as clinically benign, most likely a polymorphism.

All embryos were thawed using the Kitazato protocol, as well, and five blastocysts were sampled for PGT. PGT-A was done as per protocol for planned PGT-M embryos; however, since no causative mutation was found, further PGT-M was not done. The PGT-A report showed only two euploid blastocysts, and the remaining three to be mosaic for aneuploidy. Natural cycle frozen embryo transfer of one euploid blastocyst was done first but resulted in no implantation. A few months later, she underwent hormone replacement therapy frozen embryo transfer of the second euploid blastocyst and achieved pregnancy. Her amniocentesis chromosomal microarray genetic analysis report showed no chromosomal abnormalities. The patient's wife delivered a term baby girl.

Discussion

There are currently over thirty known gene mutations that are associated with Kallmann syndrome (KS) and congenital hypogonadotropic hypogonadism (CHH) [4, 5]. The genes tested in our patient were as follows (Table 2): ANOS1 (KAL1), AXL, CCDC141, CHD7, DUSP6, FEZF1, FGF17, FGF8, FGFR1 (KAL2), FLRT3, FSHB, GNRHR, GNRH1, HS6ST1, IL17RD, KISS1, KISS1R(GPR54), LHB, NR0B1, NSMF (NELF), POLR3B, PROK2, PROKR2, SEMA3A, SEMA3E, SOX10, SPRY4, SRA1, STS, TAC3, TACR3, WDR11.

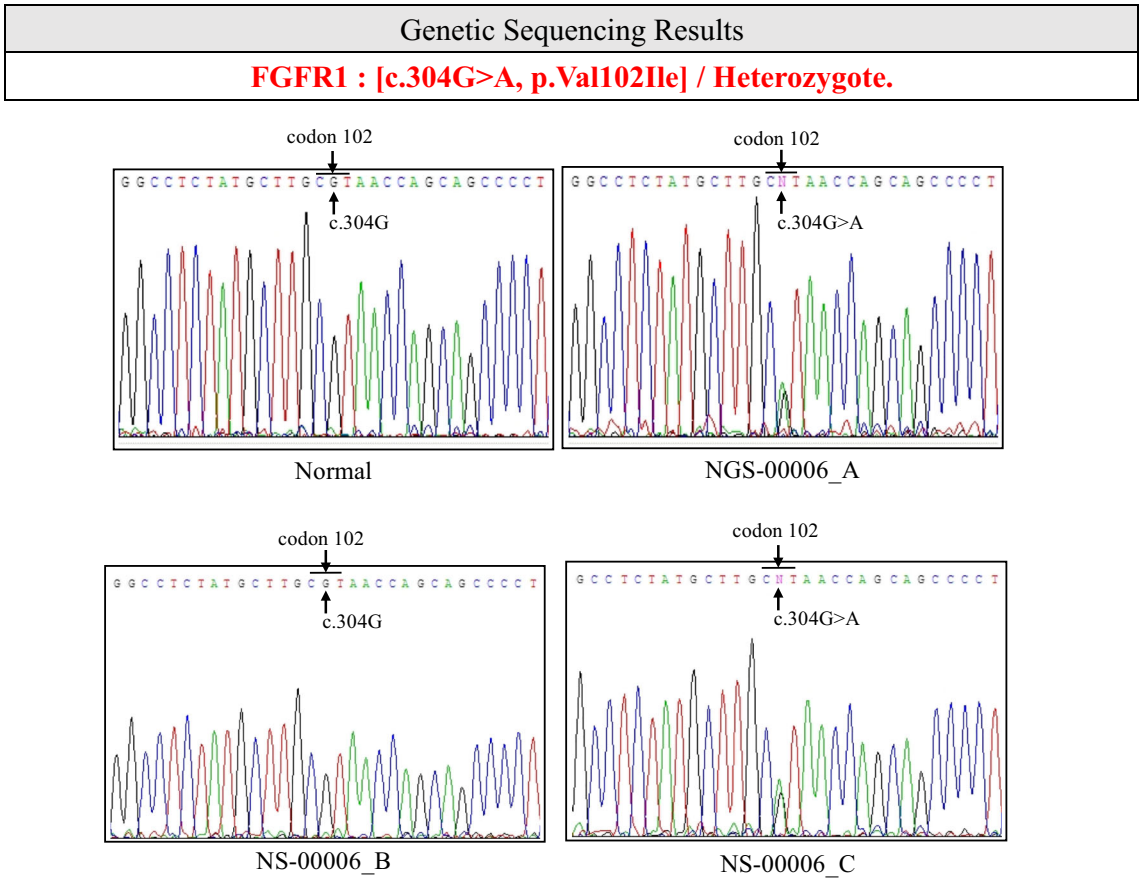

Fig. 1 Results of Sanger confirmation after targeted next-generation sequencing for FGFR1 in normal variant, the patient (A), the patient's mother (B), and the patient's father (C)

Management of the infertile Kallmann syndrome male patient requires comprehensive care. Virilization and induction of male fertility are necessary but concerns for future generations also warrant additional genetic counseling [10]. The first step of genetic counseling includes determining the mode of transmission to establish the inheritance pattern [3]. It is best if a three-generation pedigree can be created. Genetic testing can also be done, but the patient must be informed that known loci only account for roughly half of known cases, meaning genetic screening may not yield a result of a rare variant in an identified CHH or KS gene [4, 11].

In our case, the only variation found in the fibroblast growth factor receptor 1 (FGFR1) gene was of undetermined significance as listed in the Human Genome Mutation Database [9], most likely a polymorphism. Because this variation was also found in the patient's father, who is unaffected by KS or CHH, it was concluded to not be causative of the patient's condition. This suggested a de novo or sporadic mutation as the cause of KS in this case.

KS and CHH can be inherited or occur by sporadic mutation [3]. The identified modes of inheritance include X-chromosome-linked recessive, autosomal recessive, and dominant [2, 12]. Initially, this patient wished to perform PGT-M to ensure his children would not be affected. However, no specific causative mutation was found, given that the only variation found in a known gene was also found in his father who was not affected by KS. A drawback from our investigation is that WES was not done due to costs and relative difficulty in our clinical setting to perform this test for just one patient. Incomplete penetrance and variable expressivity of the disease among people with identical mutations may be observed for many CHH genes and oligogenicity is another possibility [2, 12], but it is difficult to conclude in this patient.

This case reminds clinical physicians of the importance of genetic counseling in single-gene disorder patients. In this genomic era of precision medicine, we must provide more comprehensive care for our Kallmann syndrome patients. Treatment should not stop at spermatogenesis, but continue with genetic counseling due to possible inheritance. This patient deserves further analysis with whole-genome sequencing to confirm the presence of a de novo mutation. In addition, because a de novo mutation may be

Table 2 Genes targeted by next generation sequencing with variants found listed

Gene	Accession number	Variant found
ANOS1 (KAL1)	NM_000216	---
AXL	NM_021913	---
CCDC141	NM_173648	---
CHD7	NM_017780	---
DUSP6	NM_001946	---
FEZF1	NM_001024613	---
FGF17	NM_003867	---
FGF8	NM_033163	---
FGFR1 (KAL2)	NM_023110	c.304G>A
FLRT3	NM_198391	---
FSHB	NM_000510	---
GNRHR	NM_000406	---
GNRH1	NM_000825	---
HS6ST1	NM_004807	---
IL17RD	NM_017563	---
KISS1	NM_002256	---
KISS1R (GPR54)	NM_032551	---
LHB	NM_000894	---
NR0B1	NM_000475	---
NSMF (NELF)	NM_015537	---
POLR3B	NM_018082	---
PROK2	NM_001126128	---
PROKR2	NM_144773	---
SEMA3A	NM_006080	---
SEMA3E	NM_012431	---
SOX10	NM_006941	---
SPRY4	NM_030964	---
SRA1	NM_001035235	---
STS	NM_000351	---
TAC3	NM_013251	---
TACR3	NM_001059	---
WDR11	NM_018117	---

inherited, his daughter, when of age, should be tested for KS, along with whole-genome sequencing if diagnosed. If a de novo mutation can be found and confirmed, it can become a new candidate gene, contributing to the human genome library, as a causative mutation for KS. With tools like genetic sequencing and PGT-M, inherited forms of many diseases can now be prevented from being passed on to future generations.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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