



Exploring the Genetic Diversity of Isolated Hypogonadotropic Hypogonadism and Its Phenotypic Spectrum: A Case Series

Vijay Sheker Reddy Danda *, Srinivas Rao Paidipelly, Madhavi Verepula, Piyush Lodha, Krishna Reddy Thaduri, Chaitanya Konda, Apsia Ruhi

- Department of Endocrinology, Gandhi Medical College, Hospital, Hyderabad, India

Abstract

Background: Isolated hypogonadotropic hypogonadism (IHH) is a rare disorder being classified as Kallmann syndrome (KS). The present study was conducted to study the genotype and relative proportion of different genetic mutations in IHH and to assess its correlation with phenotype.

Methods: Eleven consecutive subjects presenting to the Department of Endocrinology were retrospectively analyzed during May 2017 to December 2018 with IHH. Phenotypic features and hormonal studies were analyzed along with clinical exome by targeted gene sequencing (Next generation sequencing). Thirty-nine relevant genes were tested in the analysis.

Results: Of the 11 patients studied, five had KS and six had nIHH. At diagnosis, mean chronological age was 25 years. There were associated anomalies in KS group including bimanual synkinesia (n=2), unilateral renal agenesis (n=1) and submucosal cleft palate (n=1). Absence or hypoplasia of the olfactory bulb/sulci was found in 4/5 patients with KS. Genetic mutations in KAL1, CHD7, FGFR1, GNRHR, PROKR2, HS6ST1 genes were found in nine of the eleven subjects. Of the five subjects with KS, two had mutations in KAL1 gene. Two siblings who had bimanual synkinesia had CHD7 mutation. The genotype of nIHH subjects (n=6) was more heterogeneous.

Conclusion: This study analyzed the clinical, endocrinological, and genetic features in IHH patients. Detectable genetic mutations were seen in a large proportion of cases. A considerable heterogeneity was seen in the genotype with new variants detected. A definite correlation of phenotype-genotype was not possible, and significant overlap was seen between CHD7 and KAL1, and FGFR1 phenotypes.

Keywords: Anosmia, Genetic mutations, Hypogonadotropic hypogonadism, Kallmann syndrome, Phenotype-genotype.

To cite this article: Danda VSR, Paidipelly SR, Verepula M, Lodha P, Thaduri KR, Konda C, et al. Exploring the Genetic Diversity of Isolated Hypogonadotropic Hypogonadism and Its Phenotypic Spectrum: A Case Series. *J Reprod Infertil.* 2021;22(1):38-46. <http://dx.doi.org/10.18502/jri.v22i1.4994>.

* Corresponding Author:
Vijay Sheker Reddy Danda,
Department of
Endocrinology, Gandhi
Medical College, Hospital,
Hyderabad, India
E-mail:
drdvsreddyendo@yahoo.
com

Received: Apr. 1, 2020

Accepted: Jul. 4, 2020

Introduction

Normal pubertal development depends on the pulsatile release and action of hypothalamic gonadotropin releasing hormone (GnRH). Isolated hypogonadotropic hypogonadism (IHH) caused by defective secretion or action of hypothalamic GnRH is a rare genetic disease that presents as impaired pubertal development and infertility. When hyposmia or anosmia is seen along with hypogonadotropic hypogonadism, the condi-

tion is called Kallmann syndrome (KS). This disease induces a defect in developmental pathways and embryonic origins of olfactory and GnRH neurons. The disease associated with a normal olfaction, occurring in 45% of IHH patients, is called normosmic idiopathic hypogonadotropic hypogonadism (nIHH). Several congenital abnormalities such as midline defects (cleft lip/palate), anosmia/hyposmia, renal agenesis and bimanual synkinesia

(Or mirror movements) can be found in patients with IHH, implicated by specific gene mutation (1, 2).

IHH is genetically heterogeneous, and most cases are seen in a sporadic form. In familial cases, autosomal recessive, autosomal dominant, and X-chromosomal recessive inheritance have been described. Recently, oligogenic inheritance (Mutations in more than one IHH gene) is being recognized as contributing to incomplete penetrance and variable expressivity seen in IHH families (3). Also, families with persons having full KS, nIHH, and isolated anosmia within a single sibship have been reported (1, 3). It has been demonstrated that autosomal genes are clearly responsible for the majority of both familial and sporadic KS and that these cases have clinical and neuroendocrine phenotypes that distinguish them from the X-linked form (6).

The KS occurs due to defects in migration of GnRH and olfactory neurons. Mutations in WDR11, HS6ST1, CHD7, NELF, PROK2/PROKR2, FGFR1/FGF8, and KAL1 are associated with neuronal migration defects, leading to KS. Defects in WDR11, CHD7, PROKR2, FGF8, and FGFR1 genes are also linked to IHH, without any olfactory involvement (nIHH); however, the frequency is lower. GNRH1/GNRHR, TAC3/TACR3, and KISS1R mutations are exclusively seen in nIHH patients.

KAL1 gene is mapped to Xp22.32 chromosome. It has 14 exons. Most KAL1 mutations are nucleotide insertions or deletions which result in frameshift mutations or premature stop codons. Associated anomalies of synkinesia and unilateral renal agenesis have been suggested to be specific to the X-linked form of KS. However, synkinesia has also been reported in autosomal dominant forms of IHH as well (4). Additional phenotype-genotype correlations identified in recent studies are dental agenesis and digital bony abnormalities in FGF8/FGFR1 mutation and hearing loss in CHD7 mutation (5). Considering the rarity of IHH, this study analyzed the clinical, endocrinological, and molecular characteristics in patients with IHH.

Methods

Eleven consecutive subjects with IHH presenting to the Department of Endocrinology were analyzed during May 2017 to December 2018. Institutional ethical clearance and written informed consent was obtained from all the subjects. Base-

line characteristics of age at presentation, sex, consanguinity and family history, pubertal staging, anthropometry, anosmia and additional features were collected. The inclusion criteria for IHH included clinical signs/symptoms of hypogonadism, age >18 years, estradiol levels (<20 pg/ml), pre-pubertal testosterone (<100 ng/dL), inappropriately normal or low levels of gonadotropin, normal baseline and reserve testing of other anterior pituitary hormones (1). The GnRH agonist stimulation test was performed in all patients. After injection of leuprolide 20 µg/kg, the levels of LH and FSH at 0.2 and 4 hr were measured. The stimulated level of LH less than 5 mIU/L was suggestive of IHH. FSH, LH and gonadal steroids were analyzed in chemiluminescence immunoassay by ADVIA Centaur Siemens.

Targeted gene sequencing: Five milliliter of blood sample was collected from all the subjects, DNA was extracted and clinical exome sequencing covering hypogonadotropic hypogonadism gene panel was carried out using illumina sequencing platform. The panel included a set of 39 genes viz KAL1, CHD7, DUSP6, FEZF1, FGF17, FGF8, FGFR1, FLRT3, FSHB, GNRH1, GNRHR, HESX1, HS6ST1, IL17RD, KISS1, KISS1R, LEP, LEPR, LHB, LHX4, NR0B1, NSMF, OTUD4, PCSK1, PLXNA1, PNPLA6, PROK2, PROKR2, PROP1, RNF216, SEMA3A, SEMA3E, SEMA7A, SOX10, SOX2, SPRY4, TAC3, TACR3, and WDR11.

The results obtained were reported as per American College of Medical Genetics recommendations (7) as either variant (V), pathogenic (P), likely pathogenic (LP) and variant of uncertain significance (VUS):

1. A variant describes change in a gene that can be either benign or pathogenic.
2. Pathogenic: Disease causing genetic variation explaining the patients' clinical features.
3. The likely pathogenic variant could be pathogenic; however, the scientific evidence is insufficient to prove this conclusively. This assertion is expected to be confirmed by additional evidence.
4. Variant of uncertain significance: Variant that has been detected, but it is challenging to classify it as benign (Non-disease causing) or pathogenic (Disease causing) based on current available scientific evidence.

Mutations of clinical relevance were explained using published variants in literature and diseases databases including HGMD, ClinVar, SwissVar,

OMIM and GWAS (8-12) and common variants were filtered based on allele frequency in 1000 Genome Phase 3, ExAC, EVS, dbSNP147, 1000 Japanese Genome and internal Indian population database (13-15).

Results

Clinical characteristics: Of the eleven (n=11) cases studied, five were KS (Numbered HH1 to HH5) and six were nIHH (Numbered HH6 to HH11). Nine of the eleven subjects were male. At diagnosis, mean chronological age was 25 years, ranging between 22 years to 29 years. None of them had consanguineous parentage. Two subjects were siblings (HH3 and HH4) and none of the others had family history of hypogonadism or isolated anosmia. All the cases presented with severe reproductive phenotype and lack of secondary sexual characteristics with Tanner stage 1. All male cases had micropenis but none had any history of cryptorchidism. Subjects HH3 and HH4 had bimanual synkinesia. In addition, subject HH3 had unilateral renal agenesis while subject HH4 had submucosal cleft palate. No subject in nIHH group had any non-reproductive phenotypic features. Table 1 shows the baseline clinical characteristics of the study subjects.

Brain MRI was performed in all cases to detect the presence of an abnormal olfactory structure or other brain lesions. Absence or hypoplasia of olfactory bulb/sulci was found in four of the five KS patients. One case (HH2) had normal olfactory apparatus on MRI in spite of anosmia. No abnormality in the olfactory structures was observed in the 6 patients with nIHH; however, a Rathke's cyst in HH9 and incidentally detected microadenoma of 2 mm in HH11 were detected.

Endocrinologic profile: The GnRH agonist stimulation test with leuprolide was performed in all the cases. The baseline LH and FSH levels were 0.55 mIU/ml (Range, 0.02–1.72 mIU/ml) and 1.25 mIU/ml (Range, 0.07–2.36 mIU/ml), respectively. Peak LH was 1.51±0.66 (Range, 0.5–2.8 mIU/ml) (Table 2). The results suggest prepubertal patterns of gonadotropin secretion by GnRH stimulation.

Table 2. Endocrinological profile of the study subjects

Hormones	Results
Basal LH (mIU/ml)	0.55±0.64
Basal FSH (mIU/ml)	1.25±0.99
Peak LH (mIU/ml)	1.51±0.66
Testosterone (ng/dl)	12.6±3.8

Values are presented as mean±standard deviation

Table 1. Genotype-phenotype characteristics of the 11 study subjects

Case	Age/sex	Gene involved	Anosmia	US:LS	Arm span/height (cm)	TV/SPL	Pubertal stage	Other associated features	Peak LH	MRI olfactory bulb/brain
HH1	29/M	KAL1 (-)	Yes	0.78	173/182	3 ml/4 cm	G1P1A1	None	1.3	Absent olfactory bulbs
HH2	26/M	Contiguous deletion of KAL1 gene	Yes	0.74	171/178	3 ml/4.5 cm	G1P1A1	Gynecomastia	1.9	Normal
HH3	29/M	CHD7 (+)	Yes	0.82	175/178	1 ml/3.5 cm	G1P2A1	Synkinesia, unilateral renal agenesis	0.8	Absent olfactory bulbs
HH4	26/M	CHD7 (+)	Yes	0.83	179/180	1 ml/3 cm	G1P2A1	Synkinesia, submucosal cleft palate	1.5	Absent olfactory bulbs
HH5	26/M	No mutation	Yes	0.84	187/196	2 ml/3.5 cm	G1P2A2	None	1.2	Absent olfactory bulbs
HH6	25/M	FGFR1 (-)	No	0.73	170/180	1 ml/3 cm	G1P1A1	None	0.5	Normal
HH7	23/M	GNRHR (-)	No	0.8	155/160	2 ml/3 cm	G1P1A1	None	1.1	Normal
HH8	24/M	KAL1 (-)	No	0.92	173/184	3 ml/4 cm	G1P1A1	None	2	Normal
HH9	24/M	No mutation	No	0.76	182/185	3 ml/4 cm	G1P1A1	None	2.2	Rathke's cyst
HH10	22/F	PROKR2 (-) IL17RD (-)	No	0.81	160/166	NA	B1P1A1	None	1.4	Normal
HH11	24/F	HS6ST1	No	0.78	164/172	NA	B1P1A1	None	2.8	Microadenoma of 2 mm

TV: Testicular volume, SPL: Stretched penile length, US:LS: Upper segment to lower segment ratio, Pubertal staging (sexual maturity rating), For males: Genitalia development (G), pubic hair (P) and axillary hair (A) development. For females: Breast development (B), pubic hair (P) and axillary hair (A) development

The average basal testosterone level was 12.6 ng/dL (Range, 8–19 ng/dL) in males. The levels of testosterone in male subjects and estradiol levels in females were lower than the age and sex-matched reference range. Other anterior pituitary hormone profiles were normal in all the cases. All the cases underwent supervised treatment to assess the recovery of GnRH axis activity for 2 years and no subject had any reversal of the disease.

Molecular genetic analysis: Clinical exome sequencing results demonstrated molecular defects

in nine subjects while two did not reveal any mutation. The variations obtained were either pathogenic (1/9), likely pathogenic (4/9) or variant of uncertain significance (4/9) and were inherited either in autosomal dominant, autosomal recessive or X-linked recessive pattern. Of the detected mutations, two subjects (HH1 and HH8) had hemizygous mutation in KAL1 gene, one (HH7) had compound heterozygous mutation in GNRHR gene, while the rest had heterozygous mutations (Table 3).

Of the five subjects clinically labelled as KS,

Table 3. Genetic analysis of isolated hypogonadotropic hypogonadism cases

Case	Gene transcript	Location	Variation	Mutation type	Inheritance	Classification	gnomAD freq (%)	ExAC freq (%)	In silico analysis
HH1	KAL1 (-) (ENST00000262648)	Exon 5	c.587_594dup AGTCTGGA (p.Gln199Serfs Ter20)	Hemizygous	X-LR	P	NR	NR	Damage by MT2
HH2	contiguous deletion of exon 3 of KAL1 gene (ENST00000262648)	Exon 3	NA	NA	NA	LP	NA	NA	NA
HH3	CHD7 (+) (ENST00000423902)	Exon 2	c.1565G>T (p.Gly522Val)	Heterozygous	AD	VUS	0.2	0.2	Damage by LRT and MT2
HH4	CHD7 (+) (ENST00000423902)	Exon 2	c.1565G>T (p.Gly522Val)	Heterozygous	AD	VUS	0.2	0.2	Damage by LRT and MT2
HH5	No Mutation detected	NA	NA	NA	NA	NA	NA	NA	NA
HH6	FGFR1 (-) (ENST00000425967)	Exon 16	c.2140G>T (p.Val714Leu)	Heterozygous	AD	LP	NR	NR	Probable damage by PP2, LRT, SIFT, MT2
HH7			c.356delA (p.Tyr119PhefsTer2)	Compound heterozygous			NR	NR	Damage by MT2
	GNRHR (-) (ENST00000226413)	Exon 1	c.251C>C/A (p.Thr84Asn)	Heterozygous	AR	LP	0.0004	NR	probable damage by PP2, LRT, SIFT, and MT2
HH8	KAL1 (-) (ENST00000262648)	Exon 13	c.1955C>T (p.Thr652Met)	Hemizygous	X-LR	VUS	0.004	0.004	probable damage by PP2, LRT, SIFT, and MT2
HH9	No Mutation detected	NA	NA	NA	NA	NA	NA	NA	NA
HH10									
	PROKR2 (-) (ENST00000546004.1)	Exon 3	c.561_563dup (p.Ser188dup)	Heterozygous	AD	VUS	0.001	0.001	Damage by MT2
	IL17RD (-) (ENST00000296318.7)	Exon 7	c.676G>A (p.Gly226Ser)	Heterozygous	AD/AR	VUS	0.009	0.007	probable damage by PP2, LRT, SIFT, MT2
HH11	HS6ST1	Exon 2	c.745C>A, (p.Arg249Ser),	Heterozygous	AD	LP	0.11	0.11	Probable damage by PP2, SIFT, and MT2

XLR: X-linked recessive, AD: Autosomal dominant, AR: Autosomal recessive, P: Pathogenic, LP: Likely pathogenic, VUS: Variant of uncertain significance, NA: Not applicable, NR: Not reported, Freq: Frequencies, PP2: PolyPhen-2, MT2: Mutation Taster2, SIFT: Sorting intolerant from tolerant, LRT: Likelihood ratio test

Table 4. Proportion of reproductive and non-reproductive phenotypes

	KS (n=5)	nIHH (n=6)
Male: Female	100% male	2:1
Severe reproductive phenotype	All cases	All cases
Non-reproductive phenotype		
Bimanual synkinesia	2/5	None
Cleft lip/palate	1/5	None
Renal agenesis	1/5	None
MRI-Absent olfactory bulb	4/5	None
Mutations/variants detected		
	KAL 1-(2/5)	FGFR1-(1/6)
	CHD7-(2/5)	GNRHR-(1/6)
	No mutation detected-(1/5)	KAL1-(1/6)
		HS6ST1-(1/6)
		Digenic-PROKR2 and IL17RD (1/6)
		No mutation detected-(1/6)

KS: Kallmann Syndrome, nIHH: normosmic Idiopathic Hypogonadotropic Hypogonadism

subjects HH1 and HH2 had mutations in the KAL1 gene. In subject HH1, a hemizygous eight base pair duplication in exon 5 of the KAL1 gene, that results in a frameshift and premature truncation of the protein, was detected while in subject HH2, contiguous region corresponding to exon 3 of KAL1 gene had hemizygous deletion. Both subjects with KAL1 gene mutation had no associated features except for gynecomastia in HH2.

Subjects HH3 and HH4 were siblings and had the same mutation with autosomal dominant inheritance. A heterozygous missense variation in exon 2 of the CHD7 gene (chr8) with variant p.Gly522Val was detected in both cases. The observed variation has previously been reported along with another variant in the FGFR1 gene in a patient affected with disorder of sex development (16). The p.Gly522Val variant has a minor allele frequency of 0.06% in the South Asian population in the gnomAD database. However, the presence of phenotypic features in these cases demands consideration. Subject HH5 had no detected mutation.

The genotype of nIHH subjects (n=6) was more heterogeneous with five subjects having different mutations. In subject HH6, p.Val714Leu variant in the protein tyrosine kinase domain of the FGFR1 protein was detected. In HH7, a heterozygous single base pair deletion in exon 1 of the GNRHR, that results in frameshift and premature termination of the protein, was seen. Both variants observed in HH6 and HH7 have not been reported till date in the gnomAD and ExAC databases. In

HH8, a hemizygous missense variation in exon 13 of the KAL1 gene with variant p.Thr652Met was detected. The p.Thr652Met variant has not been reported in the gnomAD database and has a minor allele frequency of 0.005% in ExAC database. The respective case had nIHH with no phenotypic features typical of KAL1 gene mutation. The specific mutations along with their significance are shown in table 3.

Table 4 summarizes the relative proportions of reproductive, non-reproductive phenotypes, and the various genetic mutations detected.

Discussion

This study analyzed the clinical, endocrinological characteristics, and the molecular genetic spectrum of IHH which is a rare disorder with an incidence of 1-10 cases per 100,000 births (17). KS is genetically heterogeneous and most cases present as sporadic. The common genetic mutations in KS are KAL1 and FGFR1 (KAL2) account for about 15% of this condition. In this study, as mutation screening included a large number of genes known to cause IHH, molecular defects were identified in relatively large proportions of subjects. Also, mutations detected in HH1, HH6 and HH7 have not been reported in major databases till date.

Clinical characteristics: The most commonly associated abnormalities in IHH include reproductive phenotypes (Micropenis, cryptorchidism) and nonreproductive phenotypes (Anosmia/hyposmia, synkinesia, hearing loss, renal anomalies, cleft lip/palate, and dental agenesis). In our series, all the

eleven subjects had severe reproductive phenotype irrespective of genetic mutations. Other associated features differed. A French study involving 46 boys with IHH reported that cryptorchidism was detected in 69.6% (18). Importantly, placental hCG, not LH, controls androgen production during the first half of pregnancy when the trans-abdominal phase of testicular descent takes place and differentiation of male external genitalia occurs. This might explain why cryptorchidism is not found in all patients with hypogonadotropic hypogonadism like in this series. Micropenis was noted in up to 65% of males with KS by Abujbara et al., while in our series, all male cases had micropenis (19).

A coronal view of MRI is the best suited plane for overview of the olfactory tract. Olfactory bulbs are structures that lie on cribriform plate. Olfactory sulci are well seen between the gyrus rectus and the medial orbital gyrus. Abujbara et al. reported olfactory tract agenesis on MRI in 80% of cases (19/24) of KS (19). Quinton et al. indicate that a normal MRI does not rule out Kallmann syndrome as normal olfactory bulbs can be present in up to 25% of cases (20). In this study, absence or hypoplasia of olfactory bulb/sulci was found in 4 of 5 (80%) KS patients.

Genetic analysis and phenotype-genotype correlations: In isolated hypogonadotropic hypogonadism, there is clinical heterogeneity within each genetic mutation, even within the affected families. It likely indicates that the phenotype is dependent on not just the mutated gene but also other factors, which could be epigenetic factors and/or modifier genes. Oligogenic inheritance may

contribute to some extent to the partial penetrance and heterogeneity in families (22). Despite the heterogeneity, some phenotype-genotype correlations have been made from clinical observations in the patients affected by different genetic forms. Extent of hypogonadism is more variable in patients with mutations in FGF8, FGFR1, PROK2 or PROKR2, than KAL1 patients (15, 21).

Among the various non-reproductive and non-olfactory features, some cases were reported having specific genetic forms of the disease. Unilateral renal agenesis was reported in about 30% of KAL1 patients (23), but has not been reported in patients with FGFR1, FGF8, PROKR2 or PROK2 mutations. Bimanual synkinesia is highly prevalent in KAL1, seen in approximately 75% of cases (20, 24). In our case series, 3 subjects had KAL1 mutation. Two of them had anosmia with no olfactory bulbs and the other one had no abnormal phenotype. None of them had non-reproductive and non-olfactory abnormal phenotypes.

KAL1 gene mutation remains a less frequent cause of IHH. Table 5 highlights the frequency of mutations found in previous studies. Of the three mutations detected in our series including an eight base pair duplication in exon 5, a contiguous deletion of exon 3 region and a missense mutation in exon 13 are most likely to be causative mutations. Although KAL1 gene mutation is also known to cause nIHH, Bhagavath et al. found no mutations in 75 cases of IHH without anosmia/hyposmia when screened for KAL1 gene (23).

FGFR1 mutations are identified in approximately 10% of individuals with KS, with variable phenotypic expression, including cleft palate, dental

Table 5. Frequency of mutations in comparison to previous studies

Gene	Our cohort	Literature	Reference
KAL-1	3/11	6/135	Nair et al. (35)
		7/101	Oliveira et al. (6)
		2/26	Shin et al. (36)
		1/26	Vizeneux et al. (18)
FGFR1	1/11	6/135	Nair et al. (35)
		9/80	Trabach et al. (24)
		2/26	Vizeneux et al. (18)
GNRHR	1/11	9/135	Nair et al. (35)
		5/108	Beranova et al (37)
		1/26	Vizeneux et al. (18)
CHD7	2/11	2/26	Shin et al. (36)
Digenic-PROKR2 and IL17RD	1/11		

agenesis, anomalies of nasal cartilage, ear and digits. Cleft palate may occur in up to 25–30% of the cases (24). Vizeneuve et al. found heterozygous FGFR1 mutations in 2/46 boys, without any other malformation (18). Our subject with FGFR1 mutation (HH6) had no abnormal phenotype.

In PROKR2 or PROK2 gene mutation, there was a report of severe sleep disorder and marked obesity in one patient (25), which could be related to the known function of prokineticin-2 signaling in behavioral circadian rhythms, including sleep-wake and ingestive behavior (25, 26). Vizeneuve et al. reported PROKR2 mutation in 1/46 boys with normal olfaction and no other non-reproductive phenotype (18). Similarly, in our study, one of the subjects had digenic mutation, involving PROKR2 and IL17RD genes with no abnormal non-reproductive phenotype. Mutations in GnRHR gene seems to be the most frequent cause of familial IHH with normal olfaction (Nearly 40% of cases in some series), but they are rarely found (<5%) in sporadic cases (27). A GnRHR gene mutation was found in one sporadic case who had two mutations in the same gene and had no abnormal non-reproductive phenotype.

Several lines of evidence support a role for HS6ST1 as a gene associated with the pathogenesis of nIHH/KS. Tornberg et al. sequenced the coding exons of HS6ST1 from 338 GnRH-deficient patients and identified 7 subjects with sequence variants, indicating that 2% of IHH patients harbor HS6ST1 mutations (30). In our series, one case with HS6ST1 mutation was found.

Mutations were detected in large proportions of cases (9/11) in this series. Shin et al. (36) reported mutations in 5/26 cases while Vizeneuve et al. (18) reported mutations in 5/26 boys analyzed for genetic mutations.

Similarities between Kallmann syndrome and CHARGE syndrome: CHARGE syndrome is a non-random clustering of congenital anomalies including Coloboma of the eye, heart defects, Choanal Atresia, retarded growth and development, genital hypoplasia and ear abnormalities. While truncating loss-of-function mutations in CHD7 (chromo-domain helicase DNA-binding protein-7) typically cause the full CHARGE phenotype, missense mutations in CHD7 occur in both isolated KS and nIHH patients, accounting for 5 to 6% of cases (29).

It is reported that most CHARGE syndrome patients have hypogonadotropic hypogonadism and

olfactory bulb aplasia, both being the defining features of KS. Bergman et al. performed CHD7 analysis in a cohort of 36 patients with KS, and identified three heterozygous CHD7 mutations, with additional features of CHARGE syndrome, the constant feature being bilateral hearing loss (29). There has been no systematic evaluation of IHH/KS patients without a diagnosis of CHARGE syndrome for the presence of CHD7 mutations. It is hypothesized that IHH/KS could be a milder allelic variant of CHARGE syndrome. In our series, subjects HH3 and HH4 had heterozygous mutation in CHD7 gene with classic Kallmann phenotype, without any features of CHARGE syndrome.

Similarities between FGFR1 mutations and CHARGE syndrome: CHARGE syndrome shares additional traits with FGFR1 mutation, including cleft lip or palate (seen in 20–35% of FGFR1 and CHARGE patients), hypoplasia or aplasia of external ear, noted in many CHARGE patients and a few FGFR1 cases, and Coloboma, which was reported in at least one FGFR1 patient (22). In our series, two male subjects, who were siblings, had CHD7 mutation. Both of them had anosmia, absent olfactory bulbs and bimanual synkinesia. One of them had unilateral agenesis of kidney and the other had submucosal cleft palate. These features were considered typical of KAL1 and FGFR1 gene mutations, but none of our KAL1 or FGFR1 gene mutation subjects had those features while both CHD7 mutation subjects had the mentioned features. Because of the similarity between CHARGE and FGFR1 phenotypes, it could be hypothesized that CHD7 haploinsufficiency results in reduced transcription of FGFR1 or genes involved in the FGFR1 signaling pathway. Alternatively, insufficient signaling through FGFR1 could decrease CHD7 activity (31).

Recovery of hypothalamic-pituitary-gonadal (hpg) axis function can occur in few cases after normalizing the sex steroid milieu. Patients with IHH who undergo reversal can harbor IHH mutations—indicating that the effects of a genetic defect can be overcome. Considering all reports in the literature, mutations in GnRHR appear to be the leading genetic causes among cases of reversal (32, 33). Additionally, a digenic IHH case has been documented wherein a proband exhibiting reversal carries heterozygous mutations in both FGFR1 and PROKR2 (34). In our series, there was no reversal observed after a supervised follow

up of two years after beginning the treatment. Comprehensive and systematic studies on larger cohorts of reversible IHH are needed to identify genetic causes predicting reversal.

In our series, majority of patients had genetic mutations, most of them being autosomal. There was significant diversity in the mutations and phenotype. Few new variants were found as a large number of genes were analyzed. A definite correlation of phenotype-genotype was not possible, and significant overlap was seen between CHD7 and KAI1, FGFR1 phenotypes as described in previous series.

Conclusion

This study analyzed the clinical, endocrinological, and genetic features in IHH patients. However a definite correlation of phenotype-genotype was not possible. A larger series of IHH genotype and phenotype analysis might better address the issues of complexity of multiple genotypes, oligogenic inheritance and clinical heterogeneity.

Conflict of Interest

Nil.

Source of financial support: None.

References

- Boehm U, Bouloux PM, Dattani MT, de Roux N, Dodé C, Dunkel L, et al. Expert consensus document: European consensus statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol*. 2015;11(9):547-64.
- Lewkowitz-Shpuntoff HM, Hughes VA, Plummer L, Au MG, Doty RL, Seminara SB, et al. Olfactory phenotypic spectrum in idiopathic hypogonadotropic hypogonadism: pathophysiological and genetic implications. *J Clin Endocrinol Metab*. 2012;97(1):E136-44.
- Sykiotis GP, Plummer L, Hughes VA, Au M, Durani S, Nayak-Young S, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci USA*. 2010;107(34):15140-4.
- Dodé C, Levilliers J, Dupont JM, De Paepe A, Le Dû N, Soussi-Yanicostas N, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*. 2003;33(4):463-5.
- Costa-Barbosa FA, Balasubramanian R, Keefe KW, Shaw ND, Al-Tassan N, Plummer L, et al. Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. *J Clin Endocrinol Metab*. 2013;98:E943-53.
- Oliveira LM, Seminara SB, Beranova M, Hayes FJ, Valkenburgh SB, Schipani E, et al. The importance of autosomal genes in Kallmann syndrome: genotype-phenotype correlations and neuroendocrine characteristics. *J Clin Endocrinol Metab*. 2001;86(4):1532-8.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet Med*. 2015;17(5):405-24.
- Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res*. 2016;44(D1):D862-8.
- Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online mendelian inheritance in man (OMIM), a knowledgebase of human genes and genetic disorders. *Nucleic Acids Res*. 2005;33(Database issue):D514-7.
- Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, et al. The NHGRI GWAS catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res*. 2014;42(Database issue):D1001-6.
- Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, et al. Human gene mutation database (HGMDR): 2003 update. *Hum Mutat*. 2003;21(6):577-81.
- Mottaz A, David FP, Veuthey AL, Yip YL. Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. *Bioinformatics*. 2010;26(6):851-2.
- 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74.
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536(7616):285-91.
- Nagasaki M, Yasuda J, Katsuoka F, Nariyai N, Kojima K, Kawai Y, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. *Nat Commun*. 2015;6:8018.
- Ayers KL, Bouty A, Robevska G, van den Bergen JA, Juniarto AZ, Listyarsari NA, et al. Variants in congenital hypogonadotropic hypogonadism genes identified in an Indonesian cohort of 46, XY under-virilised boys. *Hum Genomics*. 2017;11(1):1.
- Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol*. 2009;5(10):569-76.

18. Vizeneuve A, Hilfiger A, Bouligand J, Pouillot M, Brailly-Tabard S, Bashamboo A, et al. Congenital hypogonadotropic hypogonadism during childhood: presentation and genetic analyses in 46 boys. *PLoS One*. 2013;8(10):e77827.
19. Abujbara MA, Hamamy HA, Jarrah NS, Shegem NS, Ajlouni KM. Clinical and inheritance profiles of Kallmann syndrome in Jordan. *Reprod Health*. 2004;1(1):5.
20. Quinton R, Duke V, de Zoysa P, Platts A, Valentine A, Kendall B, et al. The neuroradiology of Kallmann's syndrome: a genotypic and phenotypic analysis. *J Clin Endocrinol Metab*. 1996;81(8):3010-7.
21. Zenaty D, Bretones P, Lambe C, Guemas I, David M, Leger J, et al: Paediatric phenotype of Kallmann syndrome due to mutations of fibroblast growth factor receptor 1 (FGFR1). *Mol Cell Endocrinol*. 2006;254-255:78-83.
22. Hardelin JP, Julliard AK, Moniot B, Soussi-Yanicostas N, Verney C, Schwanzel-Fukuda M, et al: Anosmin-1 is a regionally restricted component of basement membranes and interstitial matrices during organogenesis: implications for the developmental anomalies of X chromosome-linked Kallmann syndrome. *Dev Dyn*. 1999;215(1):26-44.
23. Bhagavath B, Xu N, Ozata M, Rosenfield RL, Bick DP, Sherins RJ, et al. KAL1 mutations are not a common cause of idiopathic hypogonadotropic hypogonadism in humans. *Mol Hum Reprod*. 2007;13(3):165-70.
24. Trarbach EB, Costa EM, Versiani B, de Castro M, Baptista MT, Garmes HM, et al. Novel fibroblast growth factor receptor 1 mutations in patients with congenital hypogonadotropic hypogonadism with and without anosmia. *J Clin Endocrinol Metab*. 2006;91:4006-12.
25. Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, et al. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet*. 2006;2(10):e175.
26. Cheng MY, Bullock CM, Li C, Lee AG, Bermak JC, Belluzzi J, et al. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature*. 2002;417(6887):405-10.
27. Brioude F, Bouligand J, Trabado S, Francou B, Salenave S, Kamenicky P, et al. Non-syndromic congenital hypogonadotropic hypogonadism: clinical presentation and genotype-phenotype relationships. *Eur J Endocrinol*. 2010;162(5):835-51.
28. Jongmans MC, Admiraal RJ, Van Der Donk KP, Vissers LE, Baas AF, Kapusta L, et al. CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene. *J Med Genet*. 2006;43(4):306-14.
29. Bergman JE, de Ronde W, Jongmans MC, Wolfenbuttel BH, Drop SL, Hermus A, et al. The results of CHD7 analysis in clinically well-characterized patients with Kallmann syndrome. *J Clin Endocrinol Metab*. 2012;97(5):E858-62.
30. Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, Hall JE, et al. Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA*. 2011;108(28):11524-9.
31. Hardelin JP, Dodé C. The complex genetics of Kallmann syndrome: KAL1, FGFR1, FGF8, PROKR2, PROK2, et al. *Sex Dev*. 2008;2(4-5):181-93.
32. Laitinen EM, Tommiska J, Sane T, Vaaralahti K, Toppari J, Raivio T. Reversible congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations. *PLoS One*. 2012;7(6):e39450.
33. Beneduzzi D, Trarbach EB, Min L, Jorge AA, Garmes HM, Renk AC, et al. Role of gonadotropin releasing hormone receptor mutations in patients with a wide spectrum of pubertal delay. *Fertil Steril*. 2014;102(3):838-46.e2.
34. Raivio T, Sidis Y, Plummer L, Chen H, Ma J, Mukherjee A, et al. Impaired fibroblast growth factor receptor 1 signaling as a cause of normosmic idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2009;94(11):4380-90.
35. Nair S, Jadhav S, Lila A, Jagtap V, Bukan A, Pandit R, et al. Spectrum of phenotype and genotype of congenital isolated hypogonadotropic hypogonadism in Asian Indians. *Clin Endocrinol (Oxf)*. 2016;85(1):100-9.
36. Shin SJ, Sul Y, Kim JH, Cho JH, Kim GH, Kim JH, et al. Clinical, endocrinological, and molecular characterization of Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism: a single center experience. *Ann Pediatr Endocrinol Metab*. 2015;20(1):27-33.
37. Beranova M, Oliveira LM, Bédécarrats GY, Schipani E, Vallejo M, Ammini AC, et al. Prevalence, phenotypic spectrum and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2001;86(4):1580-8.