Genetics of Kallmann syndrome: Single-center Experience with Review of NGS based Genetic Studies

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Abstract

Purpose: To describe phenotype-genotype data of Asian-Indian Kallmann syndrome (KS) from our center and perform a systematic review of genetic studies using next-generation sequencing (NGS) in KS.

Methods: Seventy-eight KS probands from our center and 398 probands from published studies were included. Per-patient genetic variants were analyzed as per ACMG-guidelines. Molecular diagnosis was defined as the presence of pathogenic or likely pathogenic variant(s) in known CHH gene/s following zygosity status as per the known mode of genetic inheritance.

Result: Molecular diagnosis at our center was observed in 20.5% probands (*ANOS1*:10.2%, *FGFR1*: 6.4%, *PROKR2*: 2.5%, and *PROK2*, *SOX10*, *FGF8*, *GNRHR*: 1.3% each). Molecular diagnosis was reached more often in patients with severe than partial reproductive phenotype (28.3% vs. 4%, p=0.0013). Our center adds eight novel variants. In a per-patient systematic review (including our cohort), the molecular diagnosis was reached in 30.8%, ranging from 16.6-72.2% at different centers. The affected genes were *FGFR1* (9.6%), *ANOS1* (7.7%), *PROKR2* (6.5%), *CHD7* (4.6%), oligogenic (1.9%), *FGF8* (1%), *SOX10* (1%), and others (*PROK2*, *SEMA3A, IL17RD*, *GNRHR*:<1% each). *FGFR1* was the most commonly affected gene in most cohorts except Asia and Brazil, where *PROKR2* (in China and Japan) and *ANOS1* (in India and Brazil) were the commonest.

Conclusion(s): The global molecular diagnosis rate was 30.8% in KS cohorts whereas that in our cohort was 20.5% with a higher rate (28.3%) in those with severe reproductive phenotype. The most commonly affected gene in KS patients was *FGFR1* globally, *PROKR2* in East Asia, and *ANOS1* in India and Brazil.

1. Introduction

Kallmann syndrome (KS) is characterized by impaired olfaction in a patient with congenital hypogonadotropic hypogonadism (CHH). It is due to developmental/migrational defects of olfactory and gonadotropin-releasing hormone (GnRH) neurons. In contrast, the normosmic CHH (nCHH) is due to the defects in GnRH neuronal development, function, or action [1].

More than 40 genes are implicated in CHH pathogenesis [2]; hence, traditional Sanger sequencing may not be practically feasible and cost-effective. Moreover, prioritizing genes based on non-reproductive phenotypes (cleft lip/palate, renal agenesis, deafness, etc.) has limited utility. Advanced sequencing techniques like next-generation sequencing (NGS) enable simultaneous analysis of multiple genes of interest [3]. Such methods are less likely to miss oligogenicity, a well-described phenomenon in CHH patients. Recently, genetic studies in CHH using NGS (targeted exome panel /whole-exome sequence) have been reported worldwide.

Our center data using Sanger sequencing [4] of five genes showed that the genetic yield was 12.7% for KS and 19.6% for nCHH, which increased to 35% for nCHH using NGS analyzing 29 known CHH genes [5]. This systematic review of NGS-based studies in nCHH revealed a geographical variation in commonly affected genes [5]. This paper reports the molecular diagnostic yield using NGS and phenotype-genotype spectrum for our center's KS cohort. A systematic review of studies where multiple genes were analyzed using NGS in KS patients was also performed.

2. Methods

2.1. Patients (Our Cohort)

This study is a part of a project (EC/159/2009) entitled "genotype, phenotype and radiological correlation of idiopathic hypogonadotropic hypogonadism" approved by the Institutional Ethics Committee-II of Seth GS Medical College, Mumbai, India, and included 150 consecutive CHH probands registered between January 2009 and January 2016. Patients were enrolled after their informed consent. Here, we describe genetic data of 78 KS probands using clinical exome sequencing. The CHH diagnosis and subclassification methodology are similar to a recently published study from our center[5]. Criteria used to diagnose CHH were as follows: (1) age ≥18 years; (2) clinical symptoms and signs of hypogonadism; (3) low or normal gonadotropins along with (a) in men, morning serum total testosterone < 100 ng/dl, (b) in females with primary amenorrhoea and serum estradiol < 20 pg/ml, whenever available; (4) normal levels of other pituitary hormones; and (5) absence of any structural lesions on imaging (MRI) of the brain; and (6) no apparent systemic illness or stress. In the adolescence age group, probands were included if puberty was delayed ≥14 years in males [Testicular Volume (TV) < 4 ml] and ≥13 in females (absent breast development) with low/normal gonadotropins and low sex steroids. All these patients who presented in adolescence were followed up to 18 years of age and confirmed to have nonprogression in puberty spontaneously. If available, a detailed family history (up to three generations) was obtained. The condition was classified as familial if he/she had one or more family members with a history of a microphallus, cryptorchidism, delayed onset/completion of puberty and/or presence of other non-reproductive phenotypic features like defective sense of smell or cleft lip/palate. Probands with no affected family members were classified as sporadic. Each proband was inquired for the sense of smell, and an olfaction test was carried out using the 12 odour brief UPSIT (University of Pennsylvania smell identification test, Sensonics Inc, Cross-cultural version). A proband was classified as KS if he/she reported a reduced sense of smell on inquiry and/or a 12-item UPSIT score of ≤ 9 (0–5 were considered anosmic and 6–9 were hyposmic); otherwise, he/she was classified as nCHH. Severe reproductive phenotype is defined as a testicular volume < 4 ml in males or absent breast development in females at presentation, and partial variant is defined as a testicular volume of ≥4 ml in males or any breast development in females. Micropenis was defined as stretched penile length less than 2SD for age.

2.2 Genetic Analysis

Genomic DNA was extracted from peripheral blood using the standard method, and clinical exome sequencing was performed. The libraries were sequenced to mean > 80-100X coverage on the Illumina sequencing platform. The GATK best practices framework was followed for the identification of variants. The variants were filtered based on genes associated with CHH (29 known genes and 11 candidate genes) (supplemental data). Further nonsynonymous variants with minor allele frequency (MAF) < 0.001 as reported in the databases like 1000 Genomes and gnomAD were classified according to ACMG-2015

recommendations using the VarSome prediction tool [6]. The pathogenic (P), likely pathogenic (LP), and variants of unknown significance (VUS) variants in 29 known genes were confirmed on Sanger sequencing. Whenever possible, a segregation analysis was carried out.

2.3 Literature review

A systematic review was performed per Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines. The PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) was searched on 15 April 2022 using the following search items: ('congenital hypogonadotropic hypogonadism' OR 'idiopathic hypogonadotropic hypogonadism') AND ('mutation' OR 'genetics' OR 'next generation sequencing' OR 'whole exome sequencing'). Cohorts describing KS patients' genotype (multiple known genes) by NGS were included for analysis. Cross-references of selected publications and review articles were used to find additional studies. The PubMed search yielded 1100 articles. After exclusions (as detailed in Fig. 1), twelve study cohorts [2, 7–16] (including our present study cohort) were included for the final analysis (Supplementary data, excel sheet). We used the VarSome prediction tool (http://varsome.com) [6] to classify all the reported variants per ACMG variant classification guidelines [17]. Molecular diagnosis was defined as the presence of a P/LP variant in a known CHH gene, following the zygosity pattern as per the known mode of genetic inheritance. The monoallelic variants in the genes inherited by autosomal recessive (AR) mode were not considered for molecular diagnosis, even if the variant was P/LP. The oligogenic transmission was considered when P/LP variants in more than one known CHH gene met the molecular diagnosis criterion.

3. Statistical Analysis

Statistical analyses were performed using IBM SPSS software version 22.0 (SPSS Inc. software, Chicago, IL, USA). Fisher's exact t-test was used to compare categorical variables between two groups, and a two-sided $p < 0.05$ was considered statistically significant.

4. Results

4.1. Our Centre:

4.1.1. Phenotype:

Seventy-eight KS probands (63 males) were studied. The phenotypic features and hormonal data are detailed in table 1. The mean age at presentation was 21.5±5 years (14–36), and 53 (68%) probands had severe phenotype. Twelve male probands presented before 18 years with delayed puberty and micropenis. Three female probands presented with primary amenorrhea and hyposmia before 16 years of age. On follow-up, till 18 years, there was no spontaneous pubertal progression in these 15 probands. Sixty-three probands presented ≥ 18 years of age. Twelve probands were familial, 11 had siblings/relatives with KS, and one proband's mother had hyposmia (supplementary data). Micropenis at presentation (93.3% vs. 66.7%) and familial cases (22.6% vs. 0%) were significantly higher in the severe phenotype than the partial phenotype. There was a trend of male dominance (71.4% vs. 28.6%) and a higher proportion of patients having a history of cryptorchidism (13.3% vs. 0%) in the severe phenotype group. Serum gonadotropins and serum testosterone (in males) were significantly lower in the severe phenotype group. Molecular diagnosis was met more often (28.3% vs. 4%, $p = 0.013$) in the severe phenotype group.

4.1.2. Genotype:

Fifty-four [P/LP: 23, VUS: 13, Benign(B)/Likely Benign (LB): 18] rare nonsynonymous variants in nine known genes were detected in 36 of 78 KS probands (supplementary data). Overall the molecular diagnosis was established in 16 (20.5%) probands (*ANOS1*: 7, *FGFR1*: 3, *PROKR2*: 1, *PROK2*: 1, *GNRHR*: 1, *ANOS1 with PROKR2*: 1, *FGFR1* with *SOX10*: 1, *FGFR1* with *FGF8*: 1) (Table 2). The molecular diagnosis was established in 7 (58.3%) familial KS probands.

ANOS1: Eight male probands (severe reproductive phenotype: 8, familial: 5, synkinesia: 3, unilateral renal agenesis: 3) harbored P/LP hemizygous *ANOS1* variants (missense: 3, nonsense: 2, splice-site: 2, frameshift: 1), three of which were novel (c.1449 + 2delT, p.Leu624Arg, p.Asp252fs). One proband with p.Leu624Arg had an additional LP *PROKR2* variant (p.Val334Met). Notably, we did not find any genetic variant in two male probands (non-familial) with unilateral renal agenesis.

FGFR1: Five probands (severe reproductive phenotype: 5, males: 4, familial: 1, dental agenesis and camptodactyly: 1, congenital talipes equinovarus: 1) had monoallelic P/LP *FGFR1* variants, and three were novel (p.Gly518Asp, p.Thr726Ile, and p.Gly130Ser). A female proband with LP *FGFR1* variant (p.Gly518Asp) also had a novel monoallelic P *SOX10* variant (p.Arg178Gln). A male proband with a P *FGFR1* variant (p.Thr726Ile) also had a monoallelic LP *FGF8* variant (p.Gly151Ser).

PROKR2: A male proband (non-familial, severe phenotype) had a monoallelic LP *PROKR2* variant (p.Arg353Cys).

PROK2: A novel monoallelic missense P variant (p.Arg80Trp) was identified in a male proband with a severe reproductive phenotype, and his non-reproductive phenotype was characterized by hypertelorism and flat foot. His father and two elder sisters have isolated hyposmia, and a younger sister has KS.

GNRHR: A male proband with partial reproductive phenotype with hyposmia (UPSIT score: 3/12) and MRI brain showing aplastic olfactory bulbs (supplementary data) had a biallelic P *GNRHR* variant (p.Arg262Gln).

VUS (known CHH genes): *WDR11* (p.Arg703Gln), *WDR11* (p.Phe1150Leu), *KISS1R* (p.Gly32Cys), *HS6ST1* (p.Arg249Ser), *PNPLA6* (p.Arg433Cys), and *PROKR2* (p.Ala96Thr) monoallelic VUS were observed in seven probands. *FGFR1* (c.838 + 3A > T) with *CHD7* (p.Ser589Thr), *PNPLA6* (p.Ala823Thr) with *SOX10* (p.Gly198Asp) and *LEPR* (p.Ser1014Cys) with *FGF8* (p.Arg155Cys) digenic VUS were observed in three probands.

Candidate genes: We identified VUS in nine candidate genes (*NRP2*, *NOS1*, *NOTCH1*, *CCKBR*, *MASTL*, *JAG1*, *RD3*, *NRP2*, and *TRAPPC9*) in eighteen probands. One patient had a P *NRP2* variant (p.Ser116Ter). Five of these 19 probands met the molecular diagnosis of CHH with the affection of known CHH genes.

4.2. Results of the published literature:

Twelve study cohorts (including our center) describing KS patients' genotype (multiple known genes) by NGS were included for the analysis, and data is tabulated in Table 3. Subclassification of reproductive phenotype as severe vs. partial phenotype was not provided in the reported studies.

Molecular diagnosis was reached in 30.8% (147/476); it ranged from 16.6–72.2% across study cohorts. The affected genes were *FGFR1* (9.6%), *ANOS1* (7.7%), *PROKR2* (6.5%), *CHD7* (4.6%), FGF8 (1%), SOX10(1%) and others (*PROK2*, *IL17RD, HS6ST1, SEMA3A, GNRHR*: <1% each). FGFR1 was the most commonly affected gene in Europe, whereas *ANOS1* was commonest in India and Brazil and *PROKR2* in China. Patients *with CHD7 variants* were mostly reported from East Asia (Japan, China, South Korea, and Taiwan). *FGFR1* P/LP variants (missense: 25, splice-site: 4, frameshift: 11, and nonsense: 5) were unique except one recurrent variant (p.Gly348Arg). *ANOS1* variants (missense: 8, splice-site: 6, frameshift: 8, deletion: 3, nonsense: 10) were also unique except two recurrent variant (deletion of exon 1 and p.Met1Val). Two *PROKR2* variants were recurrent in probands from east Asia (China, Japan, and South Korea); p.Trp178Ser in 14/19 probands and p.Tyr113His in 3/19 probands while p.Leu173Arg in 7/8 probands from geographically distant regions (Europe, Brazil, and Hungary). *CHD7* P/LP variants (missense: 11, splice-site: 3, frameshift: 5, and nonsense: 2, inframe del: 1) were unique. Oligogenictity (molecular diagnosis criteria met individually for more than one gene) was seen in nine (1.9%) patients. Most common gene involved in oligogenicity was *FGFR1* (5/9).

5. Discussion

We report a molecular diagnosis rate of 20.5% in this first Asian Indian KS cohort evaluated by NGS with eight novel P/LP variants in the known CHH genes. *ANOS1* was most commonly involved contributing to half of the genetic yield. In this first systematic review of studies (including our cohort) reporting NGS-based multiple gene analysis in KS, the overall molecular diagnosis rate was 30.8% (range: 16.6%-72.2%). The affected genes were *FGFR1* (9.6%), *ANOS1* (7.7%,) *PROKR2* (6.5%), *CHD7* (4.6%), oligogenicity (1.9%), *FGF8* (1%), SOX10 (1%) and others (*PROK2*, *SEMA3A, IL17RD*, *GNRHR* :1% each). There were geographical differences in the genotype; *FGFR1* was predominant in Europe, *ANOS1* in India and Brazil, *PROKR2* in China whereas *CHD7* variants were mostly reported from East Asia.

Molecular diagnosis ranged from 16.6 to 72.2% across centers. As all the included studies in this systematic review had analyzed most of the known CHH genes, the genetic methodology is less likely to account for the variability in molecular diagnostic yield. No increment in genetic yield with the inclusion of additional genetic methods like analysis for copy number variations (CNV) further minimizes the role of genetic methods in the variability of genetic yield [7]. Other attributable factors for varied yield could be variations in cohort size, the proportion of familial cases, and the severity of reproductive phenotype. Notably, studies from (Taiwan, South Korea, and Japan) with a relatively small sample size (n < 20) had a higher yield of 41.6–72.2%, which may be partly due to ascertainment bias [8, 12, 15]. In larger KS cohorts studying adult CHH patients, molecular diagnosis yield was < 20% in China [11], and India (our center), whereas > 30% in Brazil [13], and Europe [10]. At our center, the severe reproductive phenotype was associated with a higher molecular diagnostic yield than the partial phenotype (28.3% vs. 4.0%). *ANOS1* variants, usually associated with severe CHH [18], contributed to 50% of the genetically resolved cases in our cohort, which may explain the higher genetic yield in KS patients with severe reproductive phenotype. Similarly a study from China had higher yield (45.6%) likely due to higher percentage of patients with cryptorchidism (a marker of severe phenotype) [16]. Similarly, pediatric cohorts with earlier age at CHH diagnosis due to the frequent occurrence of cryptorchidism reported a higher rate of molecular diagnosis (58.8% and 72.2%) [8, 15]. In contrast, in a few studies with involving patients younger than 18 years without cryptorchidism, the molecular diagnosis rates were relatively low (27.6% and 23.8%) [7, 9]. Although a definitive role cannot be ascertained, ethnic differences in genetic characteristics may account for the variability in the rates of molecular diagnosis among KS cohorts.

Previous studies have demonstrated oligogenic variants in the known CHH genes in up to 20% of patients [2]. In this systematic review analysis, oligogenicity was observed only in 1.9% of probands as it was defined by a stricter definition (molecular diagnosis criteria met individually for two or more genes). Most common gene associated with oligogenicity is *FGFR1*. Strict adherence to ACMG guidelines should be ensured in ascertaining a pathogenic role for a novel variant and molecular diagnosis or true oligogenicity should be considered only if P/LP variants following the zygosity pattern for known modes of inheritance are observed in a CHH patient.

Our study is the first to highlight the geographical differences in the genetics of KS. Predominantly affected genes were *FGFR1* in Europe, *ANOS1* in India and Brazil, and *PROKR2* in China. Patients with *CHD7* variants were mainly reported from East Asia (Japan, China, South Korea, and Taiwan). *PROKR2* predominance in the Chinese population can be explained by the presence of a two recurring founder variant (p.Trp178Ser and p.Tyr113His). Despite having non-recurring variants in *FGFR1*, *ANOS1*, and *CHD7*, regional differences observed in their prevalence in KS are probably due to ethnic variation.

Across studies, genetic basis remains unknown in the majority (27.8%-83.4%) of KS. Even after considering VUS following the zygosity pattern as per the known mode of genetic inheritance in a known CHH gene for molecular diagnosis, the genetic yield increased by only 11% (Table 3). Further, the addition of other genetic methods like CNV analysis also did not increase the genetic yield [7]. The genetic cause was not ascertained in 41.6% (5/12) of familial KS probands of our center. This still needs more genetic studies to identify new genetic players in the pathogenesis of KS. Taking all factors into account, a large proportion of KS may not have a monogenic/digenic genetic etiology, and rather may be governed by non-genetic unknown factors.

One of our probands harbored a biallelic P *GNRHR* variant (p.Arg262Gln) which is not previously described with KS. This proband had partial reproductive phenotype, hyposmia on the clinical history and UPSIT (score: 3/12), and rudimentary olfactory bulbs. The association of this variant in homozygous state with partial reproductive phenotype, but not with hyposmia, has been described previously [19]. We speculate that hyposmia in this proband is due to oligogenicity with a defect in an unknown CHH gene(s) or deletion in a known CHH gene.

Description of molecular diagnosis in Asian Indian KS patients by NGS and systematic collation of NGS-based genetic data of the published KS cohorts is a novel feature of this study. Verification of all the reported variants in the VarSome prediction tool to classify the variants as per ACMG criteria is a major strength of the study (Supplementary Excel). Replication of our previous finding of higher molecular diagnostic yield in severe reproductive phenotype even in KS cohort, similar to nCHH cohort, is noteworthy. A limitation of the review is that some studies could not be included due to a lack of per-patient genotype of KS probands or small cohort size (< 10).

In conclusion, the molecular diagnosis was reached in 20.5% of Asian Indian KS probands with 8 novel P/LP variants, enriching the genetic spectrum of KS. *ANOS1* was the most commonly involved gene. The systematic review of NGS-based studies revealed the molecular diagnosis in 30.8% of KS patients; *FGFR1* was the most commonly affected gene globally whereas *ANOS1* and *PROKR2* variants predominated in India and East Asia, respectively. Similar to nCHH cohort, *PROKR2* p.Trp178Ser is a recurrent variant in KS cohorts of East Asia. A large number of genetically unresolved KS cases including familial probands warrant further studies with different strategies to unravel the novel molecular mechanisms in them.

Declarations

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Contributors: ARL and TRB conceived the idea and designed the study. VAP and NSS wrote the first draft of the report with input from ARL, SA, SJ, and TRB. AVE performed the genetic study. VS, SJ, MK and SM accessed and verified the data; VAP and RS performed the statistical analysis which was verified by NSS and ARL. VAP, RS, ARL, NSS, and TRB were involved in the management of patients and data collection. NSS, ARL, SM, SA, MK and TRB supervised the entire data collection and management and provided inputs in the revision of the draft. All the authors were involved in the critical revision of the manuscript.

Declaration of interests: We declare no competing interests.

Compliance with ethical standard

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

Informed consent: Informed consent was obtained from all individual participants involved in the study.

Ethical approval: All procedures involving human participants were following the ethical standards of the institutional and/or national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by Institutional Ethics Committee III of Seth G S Medical College and KEM hospital (EC/159/2009).

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Tables

Table 1: Comparison of the severe and partial phenotype of KS at our center

Parameters	Severe phenotype n=53	Partial phenotype n=25	p-value
Male:Female	45:8	18:7	0.22
Familial cases n(%)	12 (22.6%)	0	0.0139
Micropenis at presentation n(%)	42 (93.3%)	12 (66.7%)	0.0036
Cryptorchidism n(%)	6 (13.3%)	0	0.10
Sensory Neural Hearing loss n(%)	2 (2%)	1 (0.04%)	0.96
Serum follicle stimulating hormone (IU/L) [median (IQR)]	0.61 (0.4-1.5)	1.07 (0.5-1.76)	0.000899
Serum luteinizing hormone (IU/L) [median (IQR)]	0.20 (0.07-0.49)	0.50 (0.11-1.36)	0.000036
Serum total testosterone in males (ng/ml) [median (IQR)]	0.27 (0.10-0.36)	0.35 (0.26-0.55)	0.00001
Molecular Diagnosis n(%)	15 (28.3%)	1 (4%)	0.013
Molecular Diagnosis in apparently sporadic probands n(%)	8 (19.5%)	1 (4%)	0.13

Table 2: Characteristics of KS probands having positive molecular diagnosis at our center

Gene	Patient ID	Gender	Reproductive Phenotype	Familial	Zygosity	Nucleotide Change	Protein change	Variant Type	First described DOI
ANOS1 NM_000216.3	H14	M	Severe	N	Hemizy	c.1449+2delT	-	Splice Site	Novel
	H93	M	Severe	Y	Hemizy	c.487T>C	p.Cys163Arg	Missense	10.1007/BF03346695
	H97	M	Severe	N	Hemizy	c.571C>T	p.Arg191Ter	Nonsense	10.1210/jcem.86.4.7420
	H132	M	Severe	Y	Hemizy	c.1A>G	p.M1V	Missense	10.1111/cen.13009
	146	M	Severe	N	Hemizy	c.1270C>T	p.Arg424Ter	Nonsense	10.1210/jc.2003-030476
	H165	M	Severe	N	Hemizy	c.1871T>G	p.Leu624Arg	Missense	Novel
	H220	M	Severe	Y	Hemizy	c.1984+1G>A	-	Splice Site	10.1210/jc.2014-2110
	H221	M	Severe	Y	Hemizy	c.756delC	p.Asp252fs	Frameshift	Novel
FGFR1 NM_023110.3	H30	F	Severe	N	Htz	c.533G>A	p.Gly518Asp	Missense	Novel
	H38	M	Severe	Y	Htz	c.2177C>T	p.Thr726Ile	Missense	Novel
	H75	M	Severe	N	Htz	c.2315C>T	p.Pro772Leu	Missense	10.1038/ng1122
	H100	M	Severe	N	Htz	c.443G>A	p.Arg148His	Missense	10.1111/cen.13009
	H203	M	Severe	N	Htz	c.388G>A	p.Gly130Ser	Missense	Novel
PROK2	H23	M	Severe	F	Htz	c.238C>T	p.Arg80Trp	Missense	Novel
PROKR2	H128	M	Severe	N	Htz	c.1057C>T	p.Arg353Cys	Missense	10.3881/j.issn.1000-503X.2016.01.007
	H165	M	Severe	N	Htz	c.1000G>A	p.Val334Met	Missense	10.1530/EJE-13-0419
SOX10	H30	F	Severe	N	Htz	c.533G>A	p.Arg178Gln	Missense	This study

FGF8	H38	M	Severe	N	Htz	c.451G>A	p.Gly151Ser	Missense	10.1111/cen.13009
GNRHR	H228	M	Partial	N	Hmz	c.785G>A	p.Arg262Gln	Missense	10.1056/NEJM199711273372

M-Male, F-Female, Y-Yes, N-No, Hemizy-Hemizygous, Htz-Heterozygous, P-Pathogenic, LP-Likely Pathogenic, DOI- Digital Object Identifier, ACMG- American College of Medical Genetics, ACMG Codes are as per DOI:10.1038/gim.2015.30

Table 3: Comparison of genetic yield assessing multiple known genes using next-generation sequencing in Kallmann Syndrome cohorts

Year	Country	Author	Cohort size (n) (I/Ch/Adol/Adu)	Age in years	Molecular Diagnosis n (%)	FGFR1, n (%)	ANOS1, n (%)	PROKR2, n (%)	CHD7, n (%)	Others, (n)
2014	Japan	Izumi et al	21 (0/3/7/10)	22.1±13.5	5(23.8%)	1(4.7%)	1(4.7%)	0	2(9.5%)	FGF8(1)
2017	Japan	Aoyoma et al	17 (0/4/9/4)	13.3±4.98	10 (58.8%)	3(17.6%)	3(17.6%)	1(5.8%)	3(17.6%)	
2017	China	Wang et al.	47 (1/7/39/0)	12.1±3.75	13 (27.6%)	4[#](8.5%)	2(4.2%)	6(12.7%)	2(4.2%)	PROK2[@] (1)
2018	Europe	Cassatella et al.	61	>17	26(42.6%)	12(19.6%)	1(1.6%)	4(6.5%)	4(6.5%)	FGF8(2), SEMA3A (2) SOX10(2)**, IL17RD(1)^
2018	China	Zhou et al.	77	≥17 (F) ≥18 (M)	14(18.2%)	2(2.5%)	3(3.8%)	7(9%)	2(2.5%)	-
2018	South Korea	Kim et al.	12	≥17 (F) ≥18 (M)	5(41.6%)	2(16.6%)	0	1 (8.3%)	2(16.6%)	-
2019	Brazil	Amato et al.	55	≥16 (F) ≥18 (M)	18(32.7%)	5(9%)	6(10.9%)	2(3.6%)	1(1.3%)	PROK2 (2), FGF8(1) SOX10(1)
2019	Greece	Stamou et al	30	>18	5 (16.6 %)	1 (3.3%)	3 (10%)	1(3.3%)	0	0
2020	Hungary	Butz et al.	21	30.21±12.53	6(28.5%)	3(14.2%)	2(9.5%)	1 (4.7%)	0	-
2020	Taiwan	Cho et al.	11 NA	≥13 (F) ≥14 (M)	8(72.2%)	2(18.1%)	3(27.2%)	0	2(18.1%)	HS6ST1(1)
2021	China	Zhang	46	>15 (M)	21(45.6%)	6 (13%)	5(10.9%)^^	6 (13%)	4 (8.7%)	SOX10(1), NROB1(1)^
2022	India	Our center	78 (0/0/15/63)		16(20.5%)	5(6.4%)	8(10.2%)	2^^^(2.5%)	-	PROK2 (1),SOX10^ (1) FGF8 (1)^^GNRHR (1)
Total	World		476		147(30.8%)	46 (9.6%)	37(7.7%)	31(6.5%)	22(4.6%)	PROK2 (4) ,SOX10 (5) IL17RD (1),FGF8 (5) SEMA3A (2),IL17RD (1),NROB1(1) GNRHR(1)

Infants <1 year, Children 1-10; Adolescents ≥10 and <18, Adults ≥18 year, VUS: Variant of Unknown Diagnosis, [#]:One of the probands oligogenic with *PROKR2*; [@]: Oligogenic with PROKR2; ** One of the probands oligogenic with FGF8; ^one of the probands oligogenic with FGFR1; ^^: one of the probands oligogenic with FGFR1, ^^^ :one of the probands oligogenic with ANOS1;

Figures

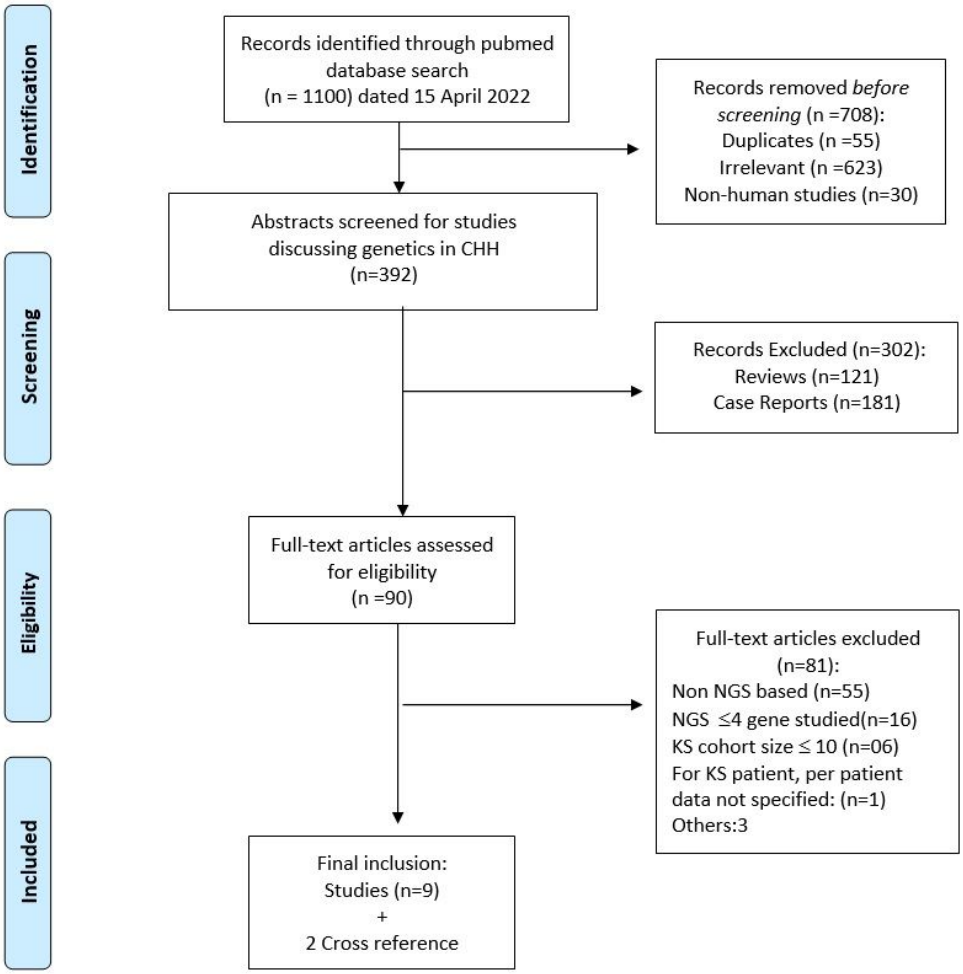

Figure 1

The PRISMA flow diagram for the systematic review

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GenotypicdataofthesystematucreviewofKS.xlsx
- SupplementalTablesandFigure.doc