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ORIGINAL ARTICLE

Analysis of PLXNA1, NRP1, and NRP2 variants in a cohort of patients with isolated hypogonadotropic hypogonadism

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Abstract

Background: Isolated hypogonadotropic hypogonadism (IHH) is a clinical syndrome described by failure of gonadal function secondary to defects on the synthesis, secretion, or action of the gonadotropin-releasing hormone (GnRH). The secreted glycoprotein SEMA3A binds its receptors NRP1 or NRP2 and PLXNA to participate in axonal projection, dendritic branching, synaptic formation, and neuronal migration. Deficiency in *SEMA3A*, *NRP1*, *NRP2*, and *PLXNA1* have been related to abnormal GnRH neuron development in mice and IHH in humans.

Methods: The aim of this study was to examine the genotypic and phenotypic spectra of the *NRP1*, *NRP2*, and *PLXNA1* genes in a large cohort of IHH probands from China. We screened *NRP1*, *NRP2*, and *PLXNA1* variants in Chinese IHH patients by whole exome sequencing and pedigree analysis.

Results: We identified 10 heterozygous missense variants in *PLXNA1*, five heterozygous missense variants in *NRP1*, and two heterozygous missense variants in *NRP2*. *NRP1* variants were found only in IHH patients with defective olfaction (i.e., Kallmann syndrome, KS). In addition, 85% (17/20) of patients harbored variants in other IHH-associated genes.

Conclusion: Our study greatly enriched the genotypic and phenotypic spectra of *PLXNA1*, *NRP1*, and *NRP2* in IHH. It may be conducive to the genetic counseling, diagnosis, and treatment of IHH with mutations in the *PLXNA1*, *NRP1*,

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and *NRP2* genes. Furthermore, our results indicated that *NRP1* were strongly linked to hearing loss.

KEYWORDS

idiopathic hypogonadotropic hypogonadism, *NRP1*, *NRP2*, *PLXNA1*, whole exome sequencing

1 | INTRODUCTION

Isolated hypogonadotropic hypogonadism (IHH) is a rare disease described by gonadal failure due to the deficiency in gonadotropin-releasing hormone (GnRH) synthesis, secretion, or action (Beate et al., 2012; Boehm et al., 2015; Topaloglu & Kotan, 2016). IHH patients are often accompanied with other developmental abnormalities, such as cleft lip and palate, tooth hypoplasia, ear deformity, congenital hearing impairment, renal hypoplasia, bilateral synkinesis, or skeletal abnormalities. About half of IHH patients have lost or low sense of smell, which is called Kallmann syndrome (KS), while IHH with normal smell is called normosic IHH (nIHH; Kim, 2015). To date, over 40 genes have been found to be associated with IHH. However, variants in all the known IHH-associated genes could only make molecular diagnosis for about 50% IHH patients.

SEMA3A, encoding Semaphorin 3A, has been proved to be one of the pathogenic genes for IHH (Hanchate et al., 2012; Kansakoski et al., 2014; Young et al., 2012). SEMA3A is essential for the development of the GnRH neurons, and loss of SEMA3A signaling alters the targeting of vomeronasal nerves and the migration of GnRH neurons into the brain, resulting in reduced gonadal size in mice (Cariboni et al., 2011). The receptors for SEMA3A, including NRP1 (OMIM accession number: 602069) or NRP2 (OMIM accession number: 602070) and PLXNA1 (OMIM accession number: 601055), are present along the vomeronasal/terminal nerve pathway, suggesting their involvement in the guidance of migrating GnRH cells (Marcos et al., 2017).

As a receptor of SEMA3A, PLXNA1 consists of extracellular signal domain (SEMA), cysteine rich motif and conserved intracellular specific plexin (SP) domain (Figure 1). Marcos et al. (2017) found that the development of

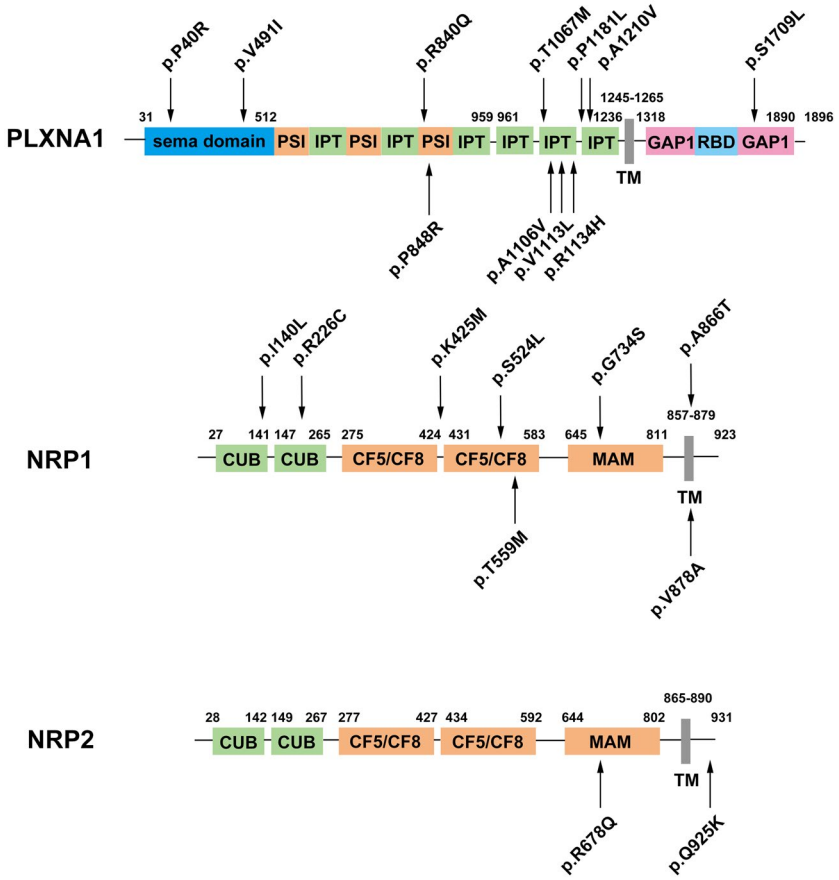

FIGURE 1 Schematic representation of the PLXNA1, NRP1, and NRP2 protein, showing the positions of the missense variants found in IHH patients. Abbreviations are as follows: sema, semaphorin domain; PSI, plexin/semaphorin/integrin domain; IPT, immunoglobulin-like fold in plexins/transcription factors; TM, transmembrane domain; GAP1, Ras GTPase-activating protein domain; RBD, Rho family GTPase-binding domain. CUB, complement components C1r and C1s/uEGF/BMP-1; CF5 and CF8, coagulation factors V and VIII; MAM, meprin/A5 antigen/receptor-like protein tyrosine phosphatase μ

peripheral olfactory system in the *Plxna1*$^{-/-}$ mutant mice was abnormal, and the migration of GnRH neurons to hypothalamus was defective, which led to the decline of reproductive ability of adult male mice. Oleari et al. (2019) found that the migration of GnRH neurons to the forebrain was reduced in the *Plxna1*$^{-/-}$/*Plxna3*$^{-/-}$ mice, and the gonadal and olfactory systems of the *Plxna1*$^{-/-}$/*Plxna3*$^{-/-}$ mice were defective. Until now, 21 *PLXNA1* variants have been identified in the IHH patients (Kotan et al., 2019; Marcos et al., 2017). And seven *PLXNA1* variants was proved to be pathogenic through functional experiments (Marcos et al., 2017).

As co-receptors for SEMA3A, NRP1/NRP2 are transmembrane proteins with a large extracellular domain, a single transmembrane domain and a very short cytoplasmic domain (Figure 1). Gu et al. (2002) found that NRP1 binds to the SEMA domain of SEMA3A and plays an important role in the repulsive axon guidance mediated by SEMA3A. Moreover, *Nrp1* mutant mice show defects in spinal cord and cranial nerve projection, and die of severe cardiovascular dysfunction in the second trimester of pregnancy (Kawasaki et al., 1999). Furthermore, Cariboni et al. (2011) found that the number of GnRH neurons in hypothalamus of *Nrp2*$^{-/-}$ mice was significantly reduced, the volume of gonad was significantly reduced, and the abnormal increase of GnRH neurons in nose of *Nrp2*$^{-/-}$ mice. Marcos et al. (2017) found three heterozygous *NRP1* missense variants and three heterozygous *NRP2* missense variants in 250 KS patients.

However, the variants of *NRP1/2* have not been reported in Asian IHH patients yet. Our aim of the current work was to investigate whether variants in *PLXNA1*, *NRP1*, and *NRP2* are present in Chinese patients with IHH.

2 | MATERIALS AND METHODS

2.1 | Patients

This study included 196 unrelated IHH patients (161 males and 35 females with a mean age at diagnosis of 21.9 years). All probands (126 with KS and 70 with nIHH) were recruited at the People's Hospital of Henan Province (Zhengzhou, China), Xiangya Hospital (Changsha, China). The patients or their adult parents signed an informed consent form. The research concerning human samples have been approved by the ethics committee of School of Life Sciences, Central South University (No. 2017030801).

IHH was diagnosed as KS or nIHH according to standard criteria (Pitteloud et al., 2002). Olfactory function, the brain, olfactory bulb structures, fundus lenses, and color atlas were examined as described previously (Dai et al., 2019). Fundus lenses and color atlas were used if necessary.

2.2 | Whole exome sequencing

Whole exome sequencing was performed on the IHH probands. The detailed whole exome sequencing methods and bioinformatics procedures have been described previously (Dai et al., 2019). ExomeDepth algorithm was used for copy number variation (CNV) analysis (Men et al., 2020). We then screened for rare sequencing variants (RSVs, <1% in the dbSNP, Genome AD, ESP6500 and 1000 Genomes database) in *PLXNA1* (GenBank accession no. NC_000003.12), *NRP1* (GenBank accession no. NC_000010.11), *NRP2* (GenBank accession no. NC_000002.12), as well as other IHH-associated genes. The detected variants were confirmed by PCR-Sanger sequencing and the sequences of PCR primers are shown in Table S1. Cosegregation analysis was conducted on family members if available.

2.3 | Bioinformatics and cosegregation analysis

Polyphen2, MutationTaster, SIFT, and Combined Annotation Dependent Depletion (CADD) were used to predict the pathogenicity of the identified variants as described previously (Dai et al., 2019). In addition, InterVar (http://wintervar.wglab.org/) was used to determine variant classification according to American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015).

3 | RESULTS

3.1 | Analysis of PLXNA1, NRP1, and NRP2 RSVs in IHH patients

Among the 196 HH patients, we identified 17 missense variants in *PLXNA1*, *NRP1*, and *NRP2*.

For *PLXNA1* (NM_032242), we identified 10 missense variants (p.P40R, p.V491I, p.R840Q, p.P848R, p.A1106V, p.T1067M, p.V1113L, p.R1134H, p.A1210V, and p.S1709L) in 11 different families, all of them are in the heterozygous state. Six variants (p.A1106V, p.T1067M, p.V1113L, p.R1134H, p.P1181L, and p.A1201V) were located in the immunoglobulin-like fold in plexins/transcription factors (IPT) domains, two variants (p.P40R and p.V491I) resided in the semaphoring (SEMA) domain, and two variants

(p.R840Q and p.P848R) was in the plexin/semaphorin/integrin (PSI) domain (Figure 1). Proband 1 inherited a p.P40R variant of *PLXNA1* and a p.Q204R variant of *OTUD4* from her unaffected father (Figure 2). Proband 5 (II:1 in family 5) carried a p.T1067M variant in *PLXNA1*, a p.G1048E variant, and a p.S1049 in *CHD7*, which was not seen in her unaffected parents (Figure 2). Proband 6 inherited a p.A1106V variant of *PLXNA1* and a p.A292E variant of *NROB1* from her unaffected mother (Figure 2). Proband 10 inherited a p.A1201V variant of *PLXNA1* from her unaffected mother, and inherited a p.T68S variant of *SPRY4* from her unaffected father (Figure 2). Proband 11 inherited a p.S1709L variant of *PLXNA1* and a p.T730I variant of *CHD7* from her unaffected mother (Figure 2). One variant (p.T1067M) is predicted to be damaging by SITF, Polyphen2, and MutaionTaster. Three variants (p.A1106V, p.A1210V, and p.S1709L) are predicted to be damaging by SITF and MutationTaster. The details of the identified *PLXNA1* variants are represented in Table 1.

For *NRP1* (NM_003873.5), eight missense variants (p.I140L, p.R226C, p.K425M, p.S524L, p.T559M, p.G734S, p.A866T, and p.V878A) were detected in eight unrelated families, all in the heterozygous state. Two variants (p.I140L and p.R226C) located in the complement components C1r and C1s/uEGF/BMP-1 (CUB) domain, two variants (p.S524L and p.T559M) were resided in the coagulation factors V and VIII (CF5/CF8) domain, two variants (p.A866T and p.V878A) located in the transmembrane (TM) domain, and one variant (p.G734S) was in the meprin/A5 antigen/receptor-like protein tyrosine phosphatase μ (MAM) domain (Figure 1). Proband 14 inherited a p.K425M variant of *NRP1* from his unaffected mother and a p.W178S variant of *PROKR2* from his unaffected father (Figure 2). Proband 15 (II:1 in family 12) carried a p.S524L variant in *NRP1*, which was not seen in her unaffected parents. He inherited a p.T340M variant of *FGFR1* from his unaffected mother and a p.R353H variant of *PROKR2* from his unaffected father (Figure 2).

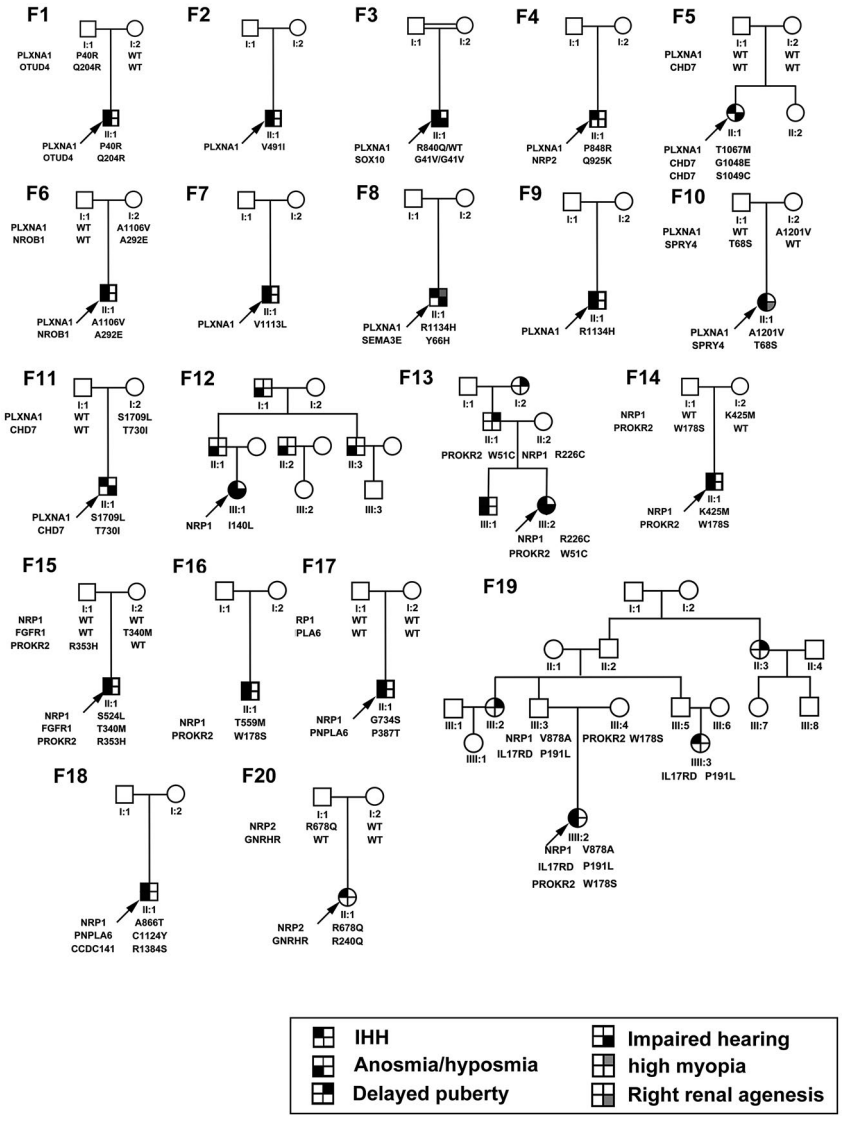

FIGURE 2 The Family analysis of IHH probands with variants in *PLXNA1*, *NRP1*, and *NRP2*. Members of the family are showed by the Roman and Arabic numerals below the symbol, and the Roman numerals denote generations. Squares, male; circles, female; arrow, the proband

TABLE 1 The prediction of PLXNA1, NRP1, and NRP2 variants using CADD, SIFT, Polyphen2, and MutaionTaster

Nucleotide change	Protein change	Inheritance	ACMG	CADD Score	SIFT	Polyphen2	MutaionTaster
PLXNA1 c.119C>G	p.P40R	N/A	LB	0.002	T	B	N
PLXNA1 c.G1471A	p.V491I	N/A	U	22.8	T	B	D
PLXNA1 c.2519G>A	p.R840Q	N/A	U	26.3	T	D	D
PLXNA1 c.C2543G	p.P848R	N/A	U	8.455	T	B	N
PLXNA1 c.C3200T	p.T1067M	N/A	U	28.8	D	D	D
PLXNA1 c.C3317T	p.A1106V	Maternal	U	24.5	D	B	D
PLXNA1 c.G3337C	p.V1113L	N/A	LB	0.351	T	B	N
PLXNA1 c.G3401A	p.R1134H	N/A	U	22.9	D	B	N
PLXNA1 c.C3629T	p.A1210V	N/A	U	23.5	D	B	D
PLXNA1 c.C5126T	p.S1709L	N/A	U	25.1	D	P	D
NRP1: c.A418C	p.I140L	N/A	U	—	D	B	D
NRP1: c.676C>T	p.R226C	Maternal	U	35	D	D	D
NRP1: c.A1274T	p.K425M	Maternal	U	—	D	D	D
NRP1 c.C1571T	p.S524L	N/A	U	—	D	P	D
NRP1 c.C1676T	p.T559M	N/A	U	25.6	T	D	D
NRP1 c.2200G>A	p.G734S	N/A	U	—	T	P	D
NRP1 c.2596G>A	p.A866T	N/A	U	—	D	P	D
NRP1 c.T2633C	p.V878A	N/A	U	25.6	D	D	D
NRP2 c.2033G>A	p.R678Q	Maternal	LB	8.318	T	B	N
NRP2 c.C2773A	p.Q925K	N/A	U	—	D	D	D

Note: ACMG criteria: P, pathogenic; B, benign; U, uncertain significance; LP, likely pathogenic; LB, likely benign. CADD, combined annotation dependent depletion; A scaled CADD score of 20 means that a variant is among the top 1% of deleterious variants in the human genome; a scaled CADD score of 30 means that the variant is in the top 0.1%. SIFT: T, tolerated; D, deleterious. PolyPhen-2: B, benign; P, possibly damaging; D, probably damaging. Mutation Taster: P, Polymorphism automatic; N, polymorphism; D, disease causing; NA, not available.

Proband 19 inherited a p.V878A variant of *NRP1* and a p.P191L variant of *IL17RD* from her unaffected father, and inherited a p.W178S variant in *PROKR2* from her unaffected mother. Her cousin carried the *IL17RD* variant (p.P191L) was diagnosed with IHH (Figure 2). Three variants (p.R226C, p.K425M, and p.V878A) are predicted to be damaging by SITF, Polyphen2 and MutaionTaster. Three variants (p.I140L, p.S524L and p.A866T) are predicted to be damaging by SITF and MutationTaster. The details of the identified *NRP1* variants are represented in Table 1.

For *NRP2* (NM_201266.1), only two heterozygous missense variants (p.R678Q and p.Q925K) were detected in two nIHH patients. The p.R678Q variant was located in the MAM domain, and the p.Q925K variant was in the intramembrane domain (Figure 1). Proband 20 inherited a p.R678Q variant of *NRP2* from her unaffected father, and she carried a p.R240Q variant in *GNRHR*, which was not seen in her unaffected parents (Figure 2). One variant (p.Q925K) is predicted to be damaging by SITF, Polyphen2 and MutaionTaster. The details of the identified *NRP2* variants are represented in Table 1.

3.2 | Additional IHH-associated variants

The results of this study showed that 17 patients had other IHH-associated variants in genes comprising of *OTUD4* (p.Q204R), *SOX10* (p.G41V), *CHD7* (p.T730I, p.G1048E, p.S1049C), *NR0B1* (p.A292E), *SEMA3E* (p.Y66H), *SPRY4* (p.T68S), *FGFR1* (p.T340M), *PROKR2* (p.W51C, p.W178S, p.R353H,), *PNPLA6* (p.P387T, p.C1124Y), *CCDC141* (p.R1384S), *IL17RD* (p.P191L), and *GNRHR* (p.R240Q). All of these variants were heterozygous, except for p.G41V in *SOX10*, which was homozygous variant in a KS patient[14], and p.A292E in *NR0B1*, which was hemizygote variant in a KS patient. The IHH-associated gene variants are represented in Table 2.

3.3 | Genotype–phenotype correlation

The clinical data of the IHH patients with *PLXNA1*, *NRP1*, and *NRP2* variants are summarized in Table 2. We have identified a *PLXNA1* variant in 11 of 196 patients (5.60%), a NRP1 variant in 8 of 196 patients (4.08%), and a NRP2

TABLE 2 Clinical information of IHH patients with PLXNA1, NRP1, and NRP2 variants

						Hormone levels				
ID	**Age**	**Sex**	**Dx**	**Nucleotide change**	**Protein change**	**LH**	**FSH**	**T**	**E2**	**Other phenotypes**
1	27	M	KS	PLXNA1 c.119C>G	p.P40R	0.48	3.66	3.17	13.07	Cryptorchidism
				OTUD4 c.A611G	p.Q204R					
2	—	M	KS	PLXNA1 c.G1471A	p.V491I	0.3	1.0	0.5	12	—
3	27	M	KS	PLXNA1 c.2519G>A	p.R840Q	0.07	0.45	0.25	<10	Impaired hearing
				SOX10 c.G122T	p.G41V					
4	26	M	nIHH	PLXNA1 c.C2543G	p.P848R	0.01	1.2	0.1	19	—
				NRP2 c.C2773A	p.Q925K					
5	16	F	nIHH	PLXNA1 c.C3200T	p.T1067M	0.04	0.3	0.41	20.81	Impaired hearing
				CHD7 c.G3143A	p.G1048E					
				CHD7 c.A3145T	p.S1049C					
6	11	M	KS	PLXNA1 c.C3317T	p.A1106V	0.4	4.61	<0.01	<0.01	—
				NR0B1 c.C875A	p.A292E					
7	19	M	KS	PLXNA1 c.G3337C	p.V1113L	0.4	1.0	0.5	29	—
8	15	M	nIHH	PLXNA1 c.G3401A	p.R1134H	0.1	0	0.5	21	Azoospermia in testis,
				SEMA3E c.196T>C	p.Y66H					High myopia
9	26	M	KS	PLXNA1 c.G3401A	p.R1134H	0.91	1.05	0.37	5.71	—
10	21	F	KS	PLXNA1 c.C3629T	p.A1210V	0.6	2.3	<20	39.5	Right renal agenesis
				SPRY4 c.A202T	p.T68S					
11	25	M	nIHH	PLXNA1 c.C5126T	p.S1709L	0.2	1.1	0.9	22	Azoospermia in testis, cubitus valgus
12	—	F	KS	NRP1: c.A418C	p.I140L	0.1	2.2	0.4	20	Impaired hearing
13	—	M	KS	NRP1: c.676C>T	p.R226C	0.22	4.2	0.3	18	Impaired hearing
				PROK2: c.153G>C	p.W51C					
14	—	M	KS	NRP1: c.A1274T	p.K425M	0.19	2.1	0.5	19	—
0				PROKR2: c.G533C	p.W178S					
15	—	M	KS	NRP1 c.C1571T	p.S524L	0.06	0.4	0.22	15	—
				FGFR1 c.1019C>T	p.T340M					
				PROKR2 c.G1058A	p.R353H					
16	—	M	KS	NRP1 c.C1676T	p.T559M	0.3	1.6	0.7	28	—
				PROKR2 c.G533C	p.W178S					
17	47	M	KS	NRP1 c.2200G>A	p.G734S	0.91	2.05	0.32	37.45	—
				PNPLA6 c.1159C>A	p.P387T					
18	25	M	KS	NRP1 c.2596G>A	p.A866T	0.13	0.83	0.2	10	Cubitus valgus, right cryptorchidism
				PNPLA6 c.3371G>A	p.C1124Y					
				CCDC141 c.4152G>C	p.R1384S					
19	11	F	KS	NRP1 c.T2582C	p.V878A	0	0.4	15.4	0.16	—
				IL17RD c.C572T	p.P191L					
				PROKR2 c.G533C	p.W178S					
20	19	F	nIHH	NRP2 c.2033G>A	p.R678Q	0.09	0.5	0.25	23.68	—
				GNRHR c.719G>A	p.R240Q					

Note: The normal ranges were as follows: FSH, 1.3–19.3 UI/L; LH, 1.2–8.6 UI/L; E2, >20 pg/mL; t, 1.75–7.81 ng/mL.

Abbreviations: Dx, diagnosis; E2, estradiol; F, female; FSH, follicle stimulating hormone; KS, Kallmann syndrome; LH, luteinizing hormone; M, male; nIHH, normosmic isolated hypogonadotropic hypogonadism;T, testosterone.

variant in 2 of 196 patients (1.02%). Through a pedigree-based analysis, 4 probands from 70 nIHH pedigrees (5.70%) and 7 probands from 126 KS pedigrees (5.50%) had a *PLXNA1* variant. Eight probands from 126 KS pedigrees (6.35%) had a *NRP1* variant. Two probands from 70 nIHH pedigrees (2.85%) had a *NRP2* variant. Other IHH-associated gene variants were found in 17 out of 20 patients (85.00%), no additional IHH-associated gene variants were found in the remaining three patients.

Proband 3 carried *SOX10* variants (p.G41V) and proband 5 carried two de novo *CHD7* variants (p.G1048E and p.S1049C) had impaired hearing (Figure 2). Proband 8 with a *SEMA3E* (p.Y66H) showed high myopia and azoospermia in testis in addition to nIHH (Figure 2). Proband 10 (II:1 in Family 10) inherited *SPRY4* (p.T68S) and *PLXNA1* (p.A1210V) variant from her unaffected father and mother, respectively. But she had right renal agenesis accompanied with KS (Figure 2). Proband 11 with a *CHD7* (p.T730I), proband 18 with a *PNPLA* (p.C1124Y) and a *CCDC141* (p.R1384S) showed cubitus valgus in addition to IHH (Figure 2). Proband 12 with a *NRP1* (p.I140L) and proband 13 with a *NRP1* (p.R226C) showed impaired hearing in addition to KS (Figure 2).

Concerning the treatment, hormone replacement therapy successfully leaded to sexual development in IHH patients of the study but failed in probands 8 and 11.

4 | DISCUSSION

In this study, we systematically analyzed the *PLXNA1*, *NRP1*, and *NRP2* RSVs in Chinese IHH patients through whole exome sequencing. In general, we identified nine novel and one previously described *PLXNA1* variants in 11 probands. Moreover, we identified seven novel and one previously described *NRP1* variants in eight probands, and two novel *NRP2* variants in two probands. The prevalence of *PLXNA1*, *NRP1*, and *NRP2* variants was 5.60%, 4.08%, and 1.02%, respectively. Although SEMA3F and PLXNA3 were recently demonstrated to be IHH-associated genes (Kotan et al., 2021), we only identified one PLXNA3 variant (A193V) whereas no SEMA3F variant was found in our cohort of 196 IHH patients.

In our study, variants in *PLXNA1*, *NRP1*, and *NRP2* can explain either the nIHH or KS phenotype. Digenic and oligogenic inheritance are quite common in IHH. Oligogenic inheritance in a patient/pedigree is thought to occur in 10–20% of all IHH cases (Boehm et al., 2015; Quaynor et al., 2011; Sykiotis et al., 2010). In our study, 85.00% probands had more than one IHH-associated gene variants. The IHH phenotype may be a results of IHH-associated gene variants. Proband 10 (II:1 in Family 10) inherited *SPRY4* (p.T68S) and *PLXNA1* (p.A1210V) variant from her unaffected father and mother, respectively. But she had right renal agenesis accompanied with KS. It illustrated the *SPRY4* and *PLXNA1* variants are enough to cause KS symptoms. Proband 14 inherited a p.K425M *NRP1* and a p.W178S *PROKR2* variant from her unaffected mother and father, respectively. It indicated the *NRP1* and *PROKR2* variants are enough to cause KS symptoms. Proband 19 inherited a p.V878A variant of *NRP1* and a p.P191L variant of *IL17RD* from her unaffected father, and inherited a p.W178S variant in *PROKR2* from her unaffected mother. It further confirmed variants in *PLXNA1*, *NRP1*, or *NRP2* may cause IHH in company with other IHH-associated gene variants (Amato et al., 2019). Therefore, we think there are yet-undiscovered IHH-associated gene variants in proband 2, 7, and 9.

In this study, we found that probands 3, 5, 12, and 13 had impaired hearing. Hearing loss was frequently found in IHH patients with mutations in *CHD7* or *SOX10*. CHD7 is a chromatin remodeling protein that controls gene expression via the formation of multi-protein complexes with specific transcription factors (Schulz et al., 2014). Autosomal dominant mutations in the CHD7 gene is associated with CHARGE syndrome. CHARGE syndrome is a nonrandom clustering of congenital anomalies including coloboma, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, ear anomalies, and deafness (Jongmans et al., 2006). Interestingly, CHD7 was enriched at the SEMA3A promotor in neural crest cells and loss of function of CHD7 leads to downregulation of SEMA3A expression (Ufartes et al., 2018). Indeed, SEMA3A was also identified as CHARGE-related gene. As a receptors of SEMA3A, NRP1 was important in the repulsive axon guidance mediated by SEMA3A (Gu et al., 2002). It will be intriguing to investigate the involvement of SEMA3A signaling in the neural development of the ear.

In conclusion, we studied the pedigree analysis and associated phenotypes of *PLXNA1*, *NRP1*, and *NRP2* variants in Chinese IHH patients, which greatly enriched the genotypic and phenotypic spectra of *PLXNA1*, *NRP1*, and *NRP2* in IHH. These results have implications for the genetic counseling, diagnosis, and treatment of IHH with mutations in the *PLXNA1*, *NRP1*, and *NRP2* genes. Meanwhile, our study indicated *NRP1* may be related to the neural development of the ear.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Meichao Men and Dan-Na Chen drafted the manuscript and figures. Xinying Wang and Ruizhi Zheng collected the IHH patient's samples. Fang Jiang and Wang Zeng were responsible for the whole exome sequencing data analysis of the IHH patients. Wenting Dai and Jia-Da Li designed the study and reviewed the manuscript.

DATA AVAILABILITY STATEMENT

Data are available if it is required due to privacy restrictions.

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