***GNRH1* Variants in Congenital Hypogonadotropic Hypogonadism: Single-Center Experience and Systematic Literature Review**

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Keywords

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Abstract

Objective: As *GNRH1* genotype-phenotype correlation in CHH is not well studied, we aim to describe the *GNRH1* variants in our CHH cohort and present a systematic review as well as genotype-phenotype analysis of all mutation-positive cases reported in the world literature. **Design:** This is a retrospective study of *GNRH1* mutation-positive patients from a western Indian center. PRISMA guidelines-based PubMed search of the published literature of all *GNRH1* mutation-positive patients was conducted. **Setting:** This study was conducted in an academic medical center. **Patient(s):** This study included 2 probands from our cohort and 19 probands from the world literature. **Main Outcome Measure(s):** Demographic details, clinical presentation, biochemistry, imaging, treatment details, and genotypic data were recorded. **Result(s):** Two probands in our cohort carried two novel pathogenic biallelic *GNRH1* variants (p.Glu24Leu, c.238-2A>G). Both had a severe reproductive phenotype. We report successful gonadotropin therapy and fertility in 1 proband. We included 19 probands from 12 studies after the

literature review. Ten CHH probands (inclusive 2 from this study) with biallelic *GNRH1* variants had severe reproductive phenotype, low gonadotropin levels, low/normal prolactin, normal pituitary imaging, and no extra-reproductive phenotype. Of seven biallelic variants reported, three were frame-shift, two were splice-site, and two were missense mutations. All of them were pathogenic/likely pathogenic without oligogenicity. Of seven monoallelic *GNRH1* variants reported in 11 probands, 4 had nonreproductive phenotype, 3 were benign/likely benign, and 4 were oligogenic. **Conclusion(s):** *GNRH1* biallelic variants lead to severe reproductive phenotype, with low gonadotropin levels without nonreproductive features or oligogenicity. However, the role of *GNRH1* monoallelic variants in CHH pathophysiology for reported variants remains questionable.

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Introduction

Congenital hypogonadotropic hypogonadism (CHH) is a genetically heterogeneous disorder with more than forty genes implicated in its pathogenesis [1]. The role of gonadotropin-releasing hormone (GnRH) in CHH was

demonstrated in 1986 when a 33.5-kb deletion in *GNRH* was identified in *hpg* mice (naturally occurring hypogonadal mice) [2]. Its role was further validated with the report of reversal of phenotype after effective gene therapy [3]. Although *GNRH1* was the most obvious candidate gene for CHH, it took over two decades to find the first human case of CHH due to a pathogenic variant [4].

GNRH1 is located on 8p21.2 and comprises four exons and three introns. Exon 1 contains the 5'-untranslated region (5'-UTR). Exon 2 encodes part of the prepro-GnRH polypeptide, consisting of a signal peptide, GnRH decapeptide, Gly-Lys-Arg cleavage site, and 23 amino acids of GnRH-associated peptide (GAP). Exon 3 encodes GAP's middle 32 amino acids, whereas exon 4 encodes GAP's last 13 amino acids and 3'-UTR [5]. Exons 3 and 4 also have elements of exonic splicing enhancers (ESEs) for pre-mRNA splicing as shown in *in vitro* studies [6, 7]. The length of GnRH1 has been evolutionally conserved, and specific residues (positions 1, 4, 9, and 10) are identical amongst vertebrates [8]. To date, only a few cases of CHH with *GNRH1* variants have been reported worldwide. Most of them harbor biallelic variants with an autosomal recessive mode of inheritance [4, 9–14]. Monoallelic variants with or without oligogenicity have also been reported [9, 15–19]. An effort to demonstrate genotype-phenotype correlation with the hypothesis of a dominant-negative role of the most common reported monoallelic variant (p.Arg31Cys) was unsuccessful [20]. Genotype-phenotype data in CHH patients with *GNRH1* variants have not been studied systematically. Analyzing the clinical, biochemical, and genetic data of such a rare condition may further our understanding of the basic physiology of the hypothalamo-pituitary-gonadal axis.

Here, we describe 2 probands with biallelic *GNRH1* pathogenic variants from a large Asian Indian CHH cohort. Additionally, a systematic literature review of the reported CHH patients harboring *GNRH1* variants published to date was done.

Methods

The present study is part of the research project No. 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; CHH index patients registered between January 2010 and January 2018 were included in the study. Inclusion and diagnostic criteria for CHH were described previously [21]. Genetic analysis was done after obtaining written informed consent. Wherever available, segregation studies were performed for the observed variant is available and alive family members of the index cases.

Demographic profile, clinical findings, hormonal profile, MRI of the pituitary, and treatment outcomes of CHH patients with *GNRH1* variants were recorded. Baseline reproductive phenotype was considered to be severe if a testicular volume was less than 4 mL in males and absent breast development in females, whereas a testicular volume ≥ 4 mL in males and some breast development in females was considered as partial reproductive phenotype [22].

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), and prolactin levels were measured by chemiluminescence immunoassay (ADVIA Centaur XP Immunoassay Systems; Siemens). Intraassay and interassay coefficients of variation were less than 10% for all the hormonal assays. Lower limits of detection for FSH, LH, T, and prolactin were 0.3 mIU/mL, 0.07 mIU/mL, 0.05 ng/dL, and 0.3 ng/mL, respectively.

Clinical exome sequencing was carried out using Illumina's Sequencing. This clinical exome panel consisted of 4,811 genes which had 29 known CHH genes. We present data of *GNRH1* variants. The targeted sequencing depth was 100X for *GNRH1*. The GATK best practices framework was followed to identify variants in the sample using Sentieon (v201808.01). The variants found were confirmed with Sanger sequencing. The functional implication of variants was predicted using *in silico* tools (Polyphen-2, Sort Intolerant from Tolerant, and MutationTaster, NNSplice, NetGene2). The minor allele frequency for the variants was checked in the databases like 1,000 Genomes and gnomAD.

GnRH Decapeptide Structure-Function Prediction Model

The full-length three-dimensional structure of the human GnRH receptor (GnRHR) was generated by homology modeling, using a template model generated by I-Tasser (<https://zhanglab.dcm.med.umich.edu/I-TASSER/>). Structural refinement was done using steepest descent and conjugate gradient methods, and its quality check was done using the Ramachandran plot (outlier: 0.7%). The GnRH1 structure was retrieved from the RCSB structural database (PDB ID: 4D5M). The variant GnRH1 (p.Gln24Leu) structure was generated using the Chimera tool (<https://www.cgl.ucsf.edu/chimera/>). Docking of GnRHR with wild-type GnRH1 and its variants was done using HADDOCK 2.0 webserver (<https://www.bonvinlab.org/software/haddock2.2>). Best-docked conformations obtained were further subjected for molecular dynamics (MD) simulation for 100 ns in the lipid bilayer using Gromacs 2018.0 software (<http://www.gromacs.org/>). The parameters for MD simulations were taken from an earlier reported similar study [23]. The stable trajectory was chosen for binding energy calculation using the MMPBSA approach (https://github.com/RashmiKumari/g_mmpbsa).

Literature Review

A systematic review of the literature was performed as per Preferred Reporting Items for Systematic Review and Meta-Analyses guidelines [24] done by 2 authors together. The PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed>) was searched on 20 April 2021 using the following search items: “Congenital Hypogonadotropic Hypogonadism” [All Fields] OR “Idiopathic Hypogonadotropic Hypogonadism” [All Fields] OR “GnRH1” [All Fields]. Studies describing CHH patients with biallelic and monoallelic *GNRH1* variants were included for analysis. Cross-references of selected publications and review articles were used to find additional studies. The PubMed search yielded 2,256 articles. After exclusions (as detailed in Fig. 1), 12 studies were included in the final

Fig. 1. The PRISMA flow diagram for the systematic review.

analysis yielding a cohort of 19 probands (4, 14–24). For the reported probands and their affected family members, demographic and phenotypic details, genetic variants, and treatment outcomes were recorded. The review was not registered. The VarSome prediction tool (<http://varsome.com>) [25] was used to classify all the reported variants as per ACMG variant classification guidelines [26].

Results

The prevalence of homozygous *GNRH1* pathogenic variants in our CHH cohort was 1.3% (2/150). It was 2.9% (2/68) for normosmic CHH (nCHH) patients. Clinical details of the 2 patients from our center are as follows.

Case 9

A boy (born of nonconsanguineous marriage) presented at 15 years of age with small-sized genitalia and

lack of other secondary sexual characteristics. He had normal olfaction, hearing, and no obvious nonreproductive phenotypic defects. He had micropenis (stretched penile length [SPL]: 3 cm), bilateral testicular volumes of 1 mL each, Tanner's pubic hair stage 2 (P2), and no gynecomastia. His serum FSH, LH, and T levels were 1.56 mIU/mL, 0.09 mIU/mL, and 0.4 ng/mL, respectively. He was started on T replacement therapy. At the last follow-up, at 18 years of age, his height was 169 cm (−0.7 SDS) with an arm span of 182 cm. His mother had attained menarche at 13 years of age; she reported having had spontaneous regular menses, unassisted conception, and two normal pregnancies. The father also had normal pubertal development. His 15-year-old younger brother lacks secondary sexual characters but has denied clinical and biochemical evaluation.

A novel splice-acceptor variant, c.238-2A>G, at intron 3-exon 4 junction was identified in a homozygous state in

the *GNRH1* gene in the proband and his younger sibling; both parents were carriers. The VarSome prediction tool classified c.238-2A>G as pathogenic (P). No variants were found in the other known CHH genes ($n = 28$).

Case 10

A 25-year-old male (born of consanguineous marriage) presented with small-sized genitalia and a lack of secondary sexual characteristics. His height was 164 cm (-1.4 SDS), with an arm span of 177 cm. He had micropenis (SPL: 3.5 cm), bilateral small testes (1 mL) with P2, but no gynecomastia. He had normal olfaction, hearing, and no obvious nonreproductive phenotypic defects. His serum FSH, LH, and T levels were 0.5 mIU/mL, <0.1 mIU/mL, and 0.15 ng/mL, respectively. His MRI brain revealed normal-sized olfactory bulbs and normal pituitary morphology. As he desired fertility, he was started on gonadotropin therapy with objectives of both pubertal induction and fertility. He was started on injection human menopausal gonadotropin (hMG) 150 units subcutaneously thrice a week and human chorionic gonadotropin (hCG) 1,000 units subcutaneously twice a week, which was increased to 2,000 units thrice a week after 6 months. His testicular volume increased from 1 mL to 8 mL each after 18 months of continued treatment. Semen analysis showed azoospermia even after 12 months of treatment, but with continued treatment, sperm count rose to 32 million/mL (50% forward progression) at 18 months after treatment. He fathered a child naturally. He had a novel missense variant (p.Gln24Leu) in the homozygous state in the *GNRH1* gene, and his parents were carriers of the same variant in the heterozygous state. The VarSome prediction tool classified this variant as likely pathogenic (LP). No variants were observed in other known CHH genes ($n = 28$). Figure 2 shows the MD simulation end structure of GnRHR (shown in gold) with wild-type GnRH-I (a) and the mutant, GnRH-I (p.Gln24Leu) (b). Binding affinity for mutant GnRH-I (p.Gln24Leu) is less due to poor interactions observed at the transmembrane helices at the outer surface. These findings are further supported by the calculated binding energy over stable MD simulation trajectory using the MMPBSA approach. The binding affinity of GnRHR-GnRH-I (p.Gln24Leu) (-91.68 ± 11.19 kcal/mol) was less than that of GnRHR-GnRH-I wild-type (-151.15 ± 8.46 kcal/mol).

Phenotype-Genotype Characteristics

A total of 19 probands harboring *GNRH1* variants (8 biallelic and 11 monoallelic) were identified in the litera-

Fig. 2. The molecular dynamics simulation end structure (obtained after 100 ns) represents atomic level interactions of GnRHR with GnRH-I (a) and GnRH-I (p.Gln24Leu) (b). The structure of GnRHR is shown in gold and GnRH-I is shown in green.

ture search. Ten CHH probands with biallelic *GNRH1* variants (including 2 from the current study) have been reported to date. History of consanguinity was present in 6, and family history with an affected sibling was reported in 4. Phenotype and genotype data were available for 13 patients (9 male probands and 4 females). Demographics, clinical findings, hormonal profile, pituitary imaging, and genotype of patients with biallelic *GNRH1* variants are summarized in Table 1. The prevalence of biallelic *GNRH1* variants in the nCHH cohorts ranges from 0.3 to 4%. The geographical regions of origin of these patients were Turkey ($n = 4$), Armenia ($n = 1$), Romania ($n = 1$), South Asia ($n = 3$), and Brazil ($n = 1$). They presented with poor pubertal development at the median age of 16 (range: 13.2–25, IQR: 14.5–18) years. All patients had a severe reproductive phenotype. Cryptorchidism was present in 33% (3/9) of males. None had any nonreproductive features like hyposmia/anosmia, midline defects, or skeletal abnormalities except case 6b who had a cleft palate. Serum FSH was <1 mIU/mL in most (12/13), whereas serum LH was <1 mIU/mL in all (13/13). None of them had high serum prolactin (10/10). A total of seven biallelic pathogenic/likely pathogenic (P/LP) variants in *GNRH1* were found in 10 probands. Of these, three were frame-shift variants (p.Leu7ThrfsTer37, p.Leu30CysfsTer12, and p.Asp41GluTer8), two were missense (p.Arg31His and p.Gln24Leu), and two were splice-site variants (c.142-2A>C and c.238-2A>G) (Fig. 3). All the reported biallelic

Table 1. Phenotype and genotypic characteristics of CHH patients with biallelic *GNRH1* variants

Case No.	Author	Cohort size (nCHH)	Age at presentation, years	Gender	Ethnicity	Familial size	Consanguinity	Reproductive phenotype	MRI pituitary	Serum biochemistry			Prolactin, ng/mL	Genotype nucleotide change	protein change	ACMG class
										FSH, mIU/L	LH, mIU/L	testosterone, ng/mL				
1a	Bouligand et al. [4]	150	18	M	Romania	Yes	No	Severe	N	0.2	0.1	0.4	–	c.18dupA (Exon 2)	p.Leu7ThrfsTer37	P
1b			17	F				Severe	N	0.1	0.17	–	3			
2	Chan et al. [9]	310	13.5	M	Armenia	No	No	Severe	NA	<0.5	<0.5	0.1	–	c.87delA (Exon 2)	p.Leu30CysfsTer12	LP
3	Mengen et al. [10]	50	15.7	M	Turkish	No	Yes	Severe*	NA	0.1	0.1	0.1	–	c.87delA/c.92G>A	p.Leu30CysfsTer12/ p.Arg31His	LP/LP
4a			19.1	M	Turkish	Yes	Yes	Severe	NA	<0.1	0.4	<0.1	–	c.92G>A (Exon 2)	p.Arg31His	LP
4b			13.7	F	Turkish			Severe	NA	<0.1	0.6	–	<5			
5	Anato et al. [11]	75	18	M	Brazilian	No	Yes	Severe*	N	<0.1	<0.1	0.11	–	c.142-2A>C Intron 2	–	P
6a	Sagi et al. [12]	Case report	17	M	Pakistani	Yes	Yes	Severe	N	<1	<1	0.08	–	c.107_110dup GAP, exon 2	p.Asp41GlufsTer8	P
6b			16	F				Severe	N	<1	<1	–	10			
7	Brachet et al. [13]	Case report	14	M	Turkish	No	Yes	Severe*	N	1.01	0.3	<0.01*	–	c.92G>A	p.Arg31His	LP
8	Turkylmaz et al. [14]	26	15	F	Turkish	No	NA	NA	NA	0.05	0.02	–	<10	c.87delA	p.Leu30CysfsTer12	P
9	This study	68	15	M	Asian Indian	Yes	No	Severe	NA	0.54	0.13	0.45	–	c.238-2A>G Intron 3	–	P
10			25	M	Asian Indian	No	Yes	Severe	N	0.5	0.1	0.15	–	c.83A>T Exon 2	p.Gln24Leu	LP

Severe reproductive phenotype: baseline TV <4 mL in males and absent breast development in females. M, male; F, female; N, normal; NA, not available; P, pathogenic; LP, likely pathogenic; ACMG, American College of Medical Genetics. * Cryptorchidism. # Level at 8 months of age.

Fig. 3. Schematic diagram of the *GNRH1* gene and distribution of reported variants.

variants are P/LP as per ACMG classification criteria. All the five exonic variants were clustered in exon 2, whereas intronic variants were observed in intron 2 and intron 3. Segregation data were available for all; parents with the variant in the heterozygous state had a normal reproductive function. p.Leu30CysfsTer12 and p.Arg31His were the two recurring variants reported from Turkey and Armenia. None had oligogenicity reported.

Characteristics of 11 CHH probands (female: 3, male: 7, gender data not reported: 1) with seven *GNRH1* monoallelic variants are shown in Table 2. The prevalence of monoallelic *GNRH1* variants in the CHH cohorts varies from 0.5 to 2%. Ethnic origin was Caucasian ($n = 6$), Asian ($n = 2$), or Latino ($n = 1$), whereas ethnicity data were not available in 2 cases. Five probands (11, 15, 17, 18, and 19) had a severe reproductive phenotype, 2 had partial (probands 12 and 13), whereas the data were not available in 4 cases. Serum FSH was ≥ 1 mIU/mL in all (5/5), and serum LH was ≥ 1 mIU/mL in 3 (3/5). Additionally, 4 probands also had nonreproductive phenotypes, hyposmia in 2 (probands 15 and 21), primary adrenal insufficiency in 1 (proband 19), and retinitis pigmentosa in 1 (proband 18). Amongst the seven variants, three (p.Glu47Asp, p.Thr58Ser, and p.Val18Met) were benign/likely benign (B/LB), two (p.Lys91Ile and p.Gly34Glu) were variants of unknown significance (VUS), and two (p.Arg31Cys, p.Arg73Ter) were P/LP. Two variants (p.Arg31Cys and p.Glu47Asp) were recurrent. Four

probands (cases 11, 16, 19, and 20) exhibited oligogenicity with additional monoallelic variants [*FGFR1* Ile239Thr (LP)/*PROKR2* Ser202Gly (LB), *FGFR1* c.622-1G>T (P), *FGFR1* splice-site (P), and *DAX1* Pro167fsTer97 (P)] (Table 2). In 5 of the 7 probands (probands 11, 12, 13, 14, 17, 19, and 21) where family segregation data were available, the carrier parent harboring the heterozygous variant had a normal reproductive function. In two families, affected mothers (patient 11 and patient 13a, p.Arg31Cys) required assistance for reproduction, whereas 1 patient had a de novo variant (p.Arg31Cys).

Discussion

We describe 2 Asian Indian CHH probands with novel *GNRH1* homozygous variants and report successful fertility with gonadotropin therapy in one. A systematic review of the reported cases with *GNRH1* variants suggests *GNRH1* biallelic variants lead to severe reproductive phenotype, with low gonadotropin levels. On the other hand, the role of reported *GNRH1* monoallelic variants in the pathophysiology of CHH remains questionable.

The novel biallelic missense variant, p.Gln24Leu, alters the first amino acid (glutamine) of GnRH1 decapeptide, which gets converted to pyroglutamate (pGlu) post-translationally. This amino acid (pGlu) is a highly conserved residue amongst vertebrates [8]. In rat studies, the

Table 2. Phenotypic and genotypic characteristic of CHH patients with monoallelic *GNRH1* variant

Case No.	Author	CHH cohort size	Age at presentation, years	Gender/ relation with proband	Ethnicity	Reproductive phenotype	Nonreproductive phenotype	Serum biochemistry			Genotype		
								FSH, mIU/L	LH, mIU/L	testosterone, ng/mL	<i>GNRH1</i> variant (ACMG class)	oligogenicity: additional monoallelic variants [#] (ACMG class)	
11	Chan et al. [9]	310	19	F	Caucasia	Severe	None	NA	NA	NA	p.Arg31Cys (LP)	<i>FGFR1</i> , p.Ile239Thr (LP) <i>PROKR2</i> , p.Ser202Gly (LB)	Familial
11a			13	Daughter	Caucasia	Severe	None	NA	NA	NA	p.Arg31Cys (LP)	<i>FGFR1</i> , p.Ile239Thr	–
11b			13	Daughter	Caucasia	Severe	None	NA	NA	NA	p.Arg31Cys (LP)	No	–
12	Maione et al. [15]	410	19	M	Caucasia	Partial	None	0.9	1	0.8	p.Arg31Cys (LP)	No	De novo
13			21	M	Caucasia	Partial	None	1.4	1	0.7	p.Arg31Cys (LP)	No	Familial
13a			65	Mother	Caucasia	NA	None	5.8	2.8	–	p.Arg31Cys (LP)	No	–
14	Quaynor et al. [16]	48	NA	F	NA	NA	None	NA	NA	NA	p.Arg31Cys (LP)	No	Carrier father
15	Amato et al. [11]	130	NA	F	Brazil	Severe	Hyposmia	NA	NA	NA	p.Glu47Asp (B)	No	NA
16	Cassatella et al. [17]	116	NA	NA	Caucasia	NA	None	NA	NA	NA	p.Glu47Asp (B)	<i>FGFR1</i> , c.622-1G>T(P)NA	
17	Chan et al. [9]	310	17.5	M	Caucasia	Severe	None	2	4	0.54	p.Arg73Ter (P)	No	Carrier father
18			16	M	Asian	Severe	Retinitis pigmentosa	NA	NA	NA	p.Thr58Ser (LB)	No	NA
19			13	M	Caucasia	Severe	Adrenal insufficiency	1.2	<0.8	0.06	p.Val18Met (LB)	<i>DAX1</i> , p.Pro167fsTer97 (P)	Carrier mother
20	Zhou et al. [18]	153	29	M	Asia	NA	None	1.09	0.42	0.1	p.Lys91Ile (VUS)	<i>FGFR1</i> splice-site, (P)	NA
21	Acierno et al. [19]	60	NA	M	NA	NA	Hyposmia	NA	NA	NA	p.Gly34Glu (VUS)	No	Carrier mother

Severe reproductive phenotype: baseline TV <4 mL in males and absent breast development in females. Partial reproductive phenotype: baseline TV ≥4 mL in males and some breast development in females. NA, not available; F, female; M, male; ACMG, American College of Medical Genetics; LP, likely pathogenic; B, benign; LB, likely benign; VUS, variant of unknown significance; P, pathogenic. [#]Targeted gene panel/multiple known CHH genes studied in all study cohorts except Chan et al. [9], where details of the number of gene studied are not explicitly described.

GnRH analog (with pGlu replaced by leucine) has very low potency (0.003%) compared to the natural GnRH decapeptide [27] which was also confirmed in the MD simulation results from our study (Fig. 2). Our patient had an nCHH phenotype with low FSH, LH, and T levels with a normal prolactin level. He responded to the gonadotrophin therapy, and fertility could be established after 18 months of treatment. Although previous reports mention sex steroid replacement therapy in CHH patients with biallelic *GNRH1*, this is the first case (case 10) of successful fertility with gonadotropin therapy in a CHH male with a biallelic *GNRH1* variant.

Mutations in *GNRH1* are a rare germline event, with very few patients reported worldwide. All the reported probands and their siblings harboring biallelic *GNRH1* variants have a severe reproductive phenotype with low gonadotropin levels (<1 mIU/mL) and cryptorchidism in 3/8 (37%) of the males (probands 3, 5, and 7). The severe phenotype suggests the reported pathogenic variants lead to poor GnRH activity. To date, seven biallelic pathogenic variants have been reported, of which two missense variants (p.Arg31His and p.Gln24Leu) occur in the GnRH decapeptide while another two variants, c.18dupA (p.Leu7ThrfsTer37) and c.87delA (p.Leu30CysfsTer12), cause frameshifts affecting the decapeptide and the GAP coding sequence. The *GNRH1* variant, p.Leu7ThrfsTer37, is shown to have undetectable GnRH activity in AtT20 pituitary cells [4]. Notably, the rest three variants (p.Asp41GlufsTer8, c.142-2A>C, and c.238-2A>G) affect only the GAP coding sequence without affecting GnRH decapeptide coding sequence and lead to a severe reproductive phenotype. Initial studies showed that GAP is an inhibitor of prolactin release [28]. Low/normal serum prolactin in all patients with biallelic *GNRH1* variants questions the inhibitory role of GAP in prolactin regulation. The splice-site variant, c.142-2A>C, has been shown to cause aberrant splicing and could lead to loss of wild-type exons 3 and 4 [11]. In the lymphocytes of the patient with this splice-site variant, an aberrant *GNRH1* transcript harboring a deletion of 4 bp (c.142-145delATAG) leading to a frameshift and a subsequent premature stop codon at amino acid position 74 was detected [11]. Another novel splice-site variant found in our patient, c.238-2A>G, could lead to loss of exon 4. How this alteration may lead to the nCHH phenotype is still unclear. A plausible explanation could be the loss of ESEs located on exon 4. These ESEs are purine-rich sequences that mediate posttranscriptional excision of the first intron of pre-mRNA. These sequences are highly similar among mice, rats, and humans. In vitro (HeLa splicing system) and in

vivo (hpg mouse) studies have shown that retention of intron 1 in the pre-mRNA affects translation of downstream open reading frame. The studies have also suggested that the splicing activity of ESE4 is much stronger than that of ESE3 [6, 7, 29]. Our novel intron 3 *GNRH1* variant with CHH phenotype also suggests the role of exon 4 in pre-mRNA processing.

Haplotype analysis has proven the founder effect for the p.Leu7ThrfsTer37 variant described in Romanian probands [4]. Recurrent variants, p.Leu30CysfsTer12 and p.Arg31His, reported from the adjacent geographical region of Turkey and Armenia also suggest the founder effect for these variants. To date, only one biallelic missense variant, p.Arg31His, has been described in Turkish patients [10].

GNRH1 monoallelic variant, p.Arg31Cys, has been hypothesized to be responsible for the phenotype of the patients in multiple studies [9, 15, 16]. This variant is reported in many families and also from the geographically common areas. The other reported monoallelic variants (p.Glu47Asp, p.Thr58Ser, and p.Val18Met) were later classified as B/LB (Table 2, probands 15, 16, 18, and 19) and were unlikely to be responsible for the pathogenesis of CHH. A proband (Table 2, proband 21) carrying a monoallelic p.Gly34Glu (VUS) variant also had hyposmia. The Kallmann phenotype suggests the involvement of the GnRH neuronal development/migration pathway, rather than GnRH secretion. Consequently, the proband (Table 2, proband 20) carrying monoallelic variant, p.Lys91Ile (VUS), also harbored a pathogenic splice-site variant in *FGFR1*, which we believe can explain the CHH phenotype by itself. The presence of the heterozygous variant may be coincidental. A proband (Table 2, proband 17) carrying a nonsense heterozygous variant, p.Arg73Ter, had inherited it from his father who had a history of delayed puberty but normal reproduction. Conventionally delayed puberty is considered as a spectrum of CHH, though recent genetic data suggest that constitutional delay in growth and puberty and CHH have distinct genetic profiles [17]. Even though the variant p.Arg31Cys is classified as LP and has been segregated positively in three unrelated families, in vitro studies have failed to confirm its dominant-negative effect [20]. Hence, the significance of the reported monoallelic variants remains unclear. Understanding the biological role of monoallelic variants needs further studies in transgenic animal models. The limitation of this systematic review includes a lack of phenotype and genetic segregation analysis data in all the CHH patients carrying monoallelic *GNRH1* variants.

To summarize, *GNRH1* mutation as a cause of CHH is a rare event (1.3% in the Asian Indian CHH cohort) suggesting a highly conserved gene structure with only a few cases described to date. We report two novel *GNRH1* variants (missense and intronic) and successful gonadotropin-based therapy in 1 proband. The novel intron 3 variant in a human subject reiterates the role of exon 4 ESEs as previously shown in in vitro and in vivo studies. CHH patients with biallelic P/LP *GNRH1* variants have severe reproductive phenotype with low gonadotropin levels, low/normal serum prolactin, and without nonreproductive phenotype, normal pituitary imaging, and low/normal serum prolactin. Amongst seven biallelic variants described to date, p.Leu30CysfsTer12 and p.Arg31His are recurring variants. The role of monoallelic *GnrRH1* variants in isolation in the pathogenesis of CHH remains questionable.

Statement of Ethics

The present study is a part of the research project No. 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. Written informed consent was obtained from the study participants. Individual patient identity was kept confidential and coded before analysis.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

N.S., V.A.P., and A.R.L. conceived the idea and designed the study. V.A.P. and N.S. wrote the first draft of the report with input from A.R.L., S.A., and T.B. A.V.E. performed genetic study. V.S., R.S., and S.S.M. accessed and verified the data. S.S.M. performed the statistical analysis which was verified by V.A.P. and V.S. V.V.B. and A.K. studied the molecular model. V.A.P., R.S., A.R.L., N.S., and T.B. were involved in management of patients and data collection. V.S., S.S.M., S.A., and T.B. supervised entire data collection and management and provided inputs in revision of the draft. All the authors were involved in critical revision of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author (A.R.L.) upon reasonable request.

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