# Results of Genetic Studies of Adolescents with Idiopathic Hypogonadotropic Hypogonadism

## Alieva DA, Mavlonov U Kh, Saydakhmedova MU, Khudaybergenov Sh A, Boboev KT and Urmanova Yu M*

*Republican Specialized Scientific and Practical Medical Center of Endocrinology of the Ministry of Health of the Republic of Uzbekistan named after academician. Y.H. Turakulova, Department of Neuroendocrinology, Tashkent Pediatric Medical Institute, Department of Endocrinology, Pediatric Endocrinology, Department of Surgical Diseases, Bukhara Regional Endocrinological Dispensary, Republican Specialized Scientific and Practical Medical Center for Hematology, Republic of Uzbekistan*

**\*Corresponding author:** Urmanova Yu M, Republican Specialized Scientific and Practical Medical Center of Endocrinology of the Ministry of Health of the Republic of Uzbekistan named after academician. Y.H. Turakulova, Department of Neuroendocrinology, Tashkent Pediatric Medical Institute, Department of Endocrinology, Pediatric Endocrinology, Department of Surgical Diseases, Bukhara Regional Endocrinological Dispensary, Republican Specialized Scientific and Practical Medical Center for Hematology, Republic of Uzbekistan

---

## ARTICLE INFO

## ABSTRACT

**Keywords:** Boys; Girls; Delayed Puberty and Growth; Gene Polymorphismgnrh1

## Background

Gonadotropin releasing hormone (GnRH) is the main hormone of the reproductive endocrine system. The existence of central hormones regulating reproduction was postulated a century ago [1]. In 1910, Crowe et al. [2]. demonstrated that disruption of the hypothalamic-pituitary connection in dogs prevents the onset of puberty. Subsequent studies led to the hypothesis that the pituitary gland is controlled by the hypothalamic factor [3-6]. However, it was not until 1971 that the amino acid sequence of GnRH was determined after extraction from the hypothalamus of thousands of pigs and sheep by the groups of Chally and Guillemin [7,8].

GnRH-secreting neurons are known to arise in the olfactory bulb and migrate to the hypothalamus [1]. Once in the hypothalamus, these GnRH neurons project axons to the median eminence and synchronize GnRH secretion in a pulsatile manner. GnRH is then transported by the portal circulation to the pituitary gland and stimulates the gonadotropins of the anterior pituitary gland, which secrete gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotropins then induce steroidogenesis and gametogenesis in the gonads. In addition to extensive physiological research on the central role of GnRH in reproduction [1], human genetic studies have highlighted the critical role of GnRH in the regulation of reproduction [9,10]. Idiopathic hypogonadotropic hypogo-

nadism (IHH) is characterized by the absence of spontaneous sexual development against the background of low levels of sex steroids and gonadotropins with normal pituitary function. When combined with anosmia, this hypogonadotropism is called Kallmann syndrome (KS), while isolated hypogonadotropic hypogonadism with normal sense of smell is called normosmic IHH (nIHH). Studies of patients with nIGG and KS have led to the identification of several genes regulating reproduction. Mutations in KAL1 [11,12], FGFR1 [13], FGF8 [14], PROK2 [15], PROKR2 and CHD7 [16] are thought to impair the development and migration of GnRH neurons, leading to KS. and/or nIGG.

Patients with mutations in PCSK1, which encodes prohormone convertase 1/3, exhibit hypogonadotropic hypogonadism due to abnormal processing of the GnRH decapeptide from its prohormone precursor [17]. Mutations in GPR54 cause nIHH by disrupting normal GnRH secretion [18,19], and mutations in GNRHR, which encodes the GnRH receptor, lead to an inability to respond to GnRH [20]. Mutations in the TAC3 and TACR3 genes, which encode neurokinin B and its receptor, respectively, have recently been implicated in nIHH [21], although their precise functions in reproduction remain unclear. The big omission from the list of genes involved in IHH is GNRH1 itself, which encodes a preprohormone that is ultimately processed to produce GnRH. The results obtained in mice strongly suggest that GNRH1 mutations in humans may cause nIHH. The hpg mouse carries a deletion of Gnrh1, which arose spontaneously and results in a complete absence of GnRH synthesis [22,23]. Male and female hpg mice are sexually infantile, infertile, and have low levels of sex steroids and gonadotropins [22]. In one of the earliest demonstrations of successful gene therapy, reproductive deficiency in hpg mice was reversed using the Gnrh1 transgene [24]. Apart from reproductive phenotypes, hpg mice appear completely normal, although dental abnormalities have recently been reported [25]. The clear association of loss of Gnrh1 function in mice with hypogonadotropic hypogonadism makes the absence of GNRH1 mutations in humans as a cause of nIHH even more puzzling.

GNRH1 is an obvious candidate gene for nIHH, so why have no mutations in GNRH1 been identified yet? [26]. One possibility is that functional mutations in genes encoding ligands occur less frequently than in genes encoding receptors due to differences in the sizes of ligands and their cognate receptors. Encoding a peptide product of only 92 amino acids, GNRH1 represents a smaller "target" for mutations than the 328 amino acids encoded by GNRHR. Indeed, for other ligand-receptor pairs involved in nIHH/KS, fewer mutations have been reported in the genes encoding the ligands (FGF8 and PROK2) than in the genes encoding the receptors [9,10]. An alternative explanation for the rarity of GNRH1 mutations is that they are rapidly eliminated from the population. This may occur due to a failure to pass on mutations to future generations, as would be expected from mutations that cause decreased fertility. Idiopathic hypogonadotropic hypogonadism (IHH) is a condition characterized by the absence

of puberty due to low levels of sex steroids and gonadotropins. IHH occurs due to abnormal secretion or action of the main reproductive hormone gonadotropin releasing hormone (GnRH). Several genes have been found to be mutated in patients with IHH, but to date no mutations have been identified in the most obvious candidate gene, GNRH1 itself, which encodes a preprohormone that is ultimately processed to form GnRH. All of the above emphasizes the relevance of this study and was the reason for it.To this end, we screened the DNA of 90 normosmic IHH (nIHH) patients and 20 healthy control subjects for GNRH1 sequence alterations.

## Purpose of the Study

Study the meaning of polymorphism GNRH 1 gene (rs 6185, rs1812594) in the development of normosmic IHH in boys and girls.

## Material and Research Methods

To achieve this goal, a genetic study was conducted in 90 adolescents diagnosed with iHH, selected during screening in pilot regions of the Republic of Uzbekistan as part of an applied project in the period June-August 2023: Kashkadarya, Jizzakh, Surkhandarya, Namangan regions and the Republic of Karakalpakstan. Among the 90 individuals, there were 73 boys and 17 girls, with an average age of 14.3 years. Disease diagnoses were made in accordance with the latest clinical guidelines. The diagnosis of nIHH was based on the absence of spontaneous puberty and low levels of sex steroids (testosterone <3.4 nmol/L in boys; estradiol, <73 pmol/L in girls) against the background of normal or unreliably low levels of gonadotropins and intact sense of smell.

### Patients were Divided into 2 Groups

- Group 1 – patients with nIH + grade 1-2 diffuse goiter with hypothyroidism – 42 patients.

- Group 2 – patients with nIHH – 48 patients.

The control group consisted of 20 healthy individuals of the corresponding average age (10 m boys and 10 girls). All 90 patients underwent a range of studies, including the study of endocrine status, general clinical, biochemical, hormonal (STH, LH, FSH, prolactin, TSH, testosterone, cortisol, free thyroxine, etc.) - in the laboratory of hormonal studies of the Republican Scientific Research and Medical Center of Endocrinology of the Ministry of Health of the Republic of Uzbekistan. In addition, they carried out X-ray (x-ray of the hand and sella turcica, CT/MRI of the sella turcica and adrenal glands in all patients, ultrasound of the genital organs), anthropometric studies (height, weight, height and weight deficit, target height, centile, growth velocity, SDS of height and weight, etc. .) based on the Tanner-Whitehouse international height-weight chart, assessment of the stage of sexual development according to Tanner, karyotyping and other studies. All genetic studies were performed NDC Immunogen test at the Institute of Human Immunology and Genomics of

the Academy of Sciences of the Republic of Uzbekistan on the basis of cooperation agreement No. 10 dated December 16, 2021 with the RSNPME Ministry of Health of the Republic of Uzbekistan on the topic "Study of the role of genetic markers PRPP-1, HS6St1, LEP, GNRH-1, Kal-1 in children with endocrine diseases". The material for the study was venous blood samples collected in vacuum tubes with EDTA as an anticoagulant. DNA extraction from peripheral blood was carried out using a commercial reagent kit "Ampli Prime RIBO-prep" (Inter-labservice LLC, Russia), according to the manufacturer's instructions.

Testingpolymorphism GNRH1 rs 6185, rs1812594 in the format Real-Time on a Rotor-Gene Q device (Quagen, Germany) using a commercial test kit from Synthol LLC (Russia) in accordance with the manufacturer's instructions. Statistical processing of the results was performed using the standard OpenEpi V.9.2 application package. The distribution of alleles and genotypes corresponded to the Hardy-Weinberg distribution law (HW). The odds ratio (OR) was calculated to describe the relative risk of developing the disease. OR>1 was considered as a positive association (predisposition) of an allele or genotype with a disease, and OR<1 ($p<0.05$) as a negative association.

## Research Results and Discussion

Table 1 shows the distribution of the selected 90 patients by age and stage of puberty. As can be seen from Table 1, the most common patients among the examined patients were aged 14.7 years - 22 boys and 8 girls (IV stage according to Tanner by age). In this case, the stage of puberty upon examination corresponded to II both 22 boys (30.1%) and 8 girls (47%). In general, when assessing the stage of puberty, it was revealed that 30 adolescents had delayed puberty, that is, puberty corresponded to stage 2 at the age of 13-14 years in both boys and girls. Next, we calculated average anthropometric indicators (Table 2). As can be seen from Table 2, the average height and weight in both groups differed significantly from the data in the control group ($p < 0.05$). At the same time, in group 1 of patients with nIH+ hypothyroidism, the lowest average values of height and weight were observed in comparison with group 1 of patients with nIHH. The next step in our research was to study hormonal disorders (Table 3). As follows from Table 3, the studied patients had a significant decrease in basal values of LH, FSH ($p < 0.05$) compared to the control group, as well as significantly low levels of free testosterone (fT) in blood plasma ($p < 0.05$) at background normoprolactinemia. Thus, the patients were diagnosed with hypogonadotropic hypogonadism.

**Table 1:** Distribution of selected 90 patients by age, gender and 5 stages of puberty according to J Tanner.

| Age, years, | Total by chronological age | Total by stages of puberty according to Tanner according to HB | Total by stages of puberty according to Tanner according to examination data |
|---|---|---|---|
| **Boys, n=73** | | | |
| 10 ± 0.5 years | - | I | - |
| 11.7 ± 0.6 years | 10 (13.7%) | II | I |
| 13.2 ± 0.8 years | 16 (21.9%) | III | I |
| 14.7 ± 0.6 years | 22 (30.1%) | IV | II |
| 15.5 ± 0.7 years | 25 (34.2%) | V | III |
| **Girls, n=17** | | | |
| 10 ± 0.5 years | - | I | - |
| 11.7 ± 0.6 years | - | II | - |
| 13.2 ± 0.8 years | 3 (17.6%) | III | I |
| 14.7 ± 0.6 years | 8 (47%) | IV | II |
| 15.5 ± 0.7 years | 6 (35.4%) | V | III |

**Table 2:** Average anthropometric indicators of patients by groups.

| Indicators | control N=20 | 1 g N=42 | 2 g N= 48 |
|---|---|---|---|
| Height, cm | 162.6 ± 17.34 160.9 ± 16.5 | 144.5± 19.6* 143.5 ± 17.3* | 150.3± 22.4* 151.2± 20.5* |
| Weight, kg | 51.32 ± 7.30 48.18 ± 7.34 | 40.9± 9.4* 38.3± 8.42* | 43.5± 8.7* 39.6± 9.6* |
| Growth deficiency | - | 18.5± 3.8 17.7± 3.9 | 12.6± 4.6 12.7± 3.4 |
| Weight deficiency | - | 12.86±0.9 10.3± 3.6 | 10.5±0.6 9.3± 2.4 |
| Growth SDS | 6.6 ± 1.2 | -1.5 | -1.6 |
| SDS weights | 3.5 ± 1.3 | -1 | -1.1 |

| Centile | 50 | 25 | 50 |
|---|---|---|---|
| Average parental height | 168.4±4.2 | 167.5±5.3 | 169.9± 3.5 |
| Designed height | 171.4±4.2 | 170.5±5.3 | 174.9± 3.5 |
| Average age | 12.5 | 9.6 ± 0.3 | 8.90±0.5 |
| Bone age | 12.5 | 5.16±0.1 | 7.96±0.4 |
| BA/ChA | 1 | 0.53±0.02 | 0.75±0.03 |

Note: Numerator - boys, denominator - girls, * - significance of differences compared to control, where * is p < 0.05; BA – bone age, ChA- chronological age.

**Table 3:** Average hormone values in the boys studied.

| Hormones | Control n= 10 | Boys n= 73 | |
|---|---|---|---|
| STH | 3.9±0.2ng/ml | 1.3± 0.4 | p >0.05 |
| IGF-1 | 156.5±9.8 ng/ml | 119.8±12.7 | p <0.05 |
| LH | 5.2±0.3 mIU/L | 1.21 ±0.3 | p <0.05 |
| FSH | 5.3±0.1 mIU/L | 1.4±0.5 | p <0.05 |
| TSH | 2.5±0.2mIU/L | 1.82±0.7 | p >0.05 |
| Prolactin | 5.7±0.3 ng/ml | 4.4 ±0.8 | P >0.05 |
| Free Testosterone | 12.6 ±1.6 nmol/l | 3.9±0.2 | p <0.05 |
| Cortisol | normal morning 596.5 ± 11.7 nmol/l | 589.25±9.3 | p >0.05 |
| Free thyroxine | 15.8 ± 0.9 pmol/l | 15.4± 1.4 | p >0.05 |

Note: P - significance of differences compared to the control group (P <0.05). The table for comparison shows fluctuations in hormone levels from 11 to 16 years of age in the control group (healthy individuals).

Table 4 gives average hormone values in the studied girls on the 14th day of the cycle. As follows from Table 4, on the 14th day of the cycle, the studied patients had a significant decrease in basal values of LH, FSH (p < 0.05) compared to the control group, as well as significantly low levels of E2 in the blood plasma (p < 0.05) against the background normoprolactinemia. Thus, the patients were diagnosed with hypogonadotropic hypogonadism. Next, we performed calculations for genetic studies (Table 5). The control group consisted of 20 healthy children of the corresponding age (10 boys and 10 girls) with the T/T genotype. Among the examined individuals, the T/T genotype was detected in 64 (71%) patients, the T/C genotype in 22x (24.4%) and 4 (4.4%) had a mutation of the C/C genotype. It should be noted that in 5 (6.6%) cases the rs 6185 polymorphism of the GNRH 1 gene was found. Polymorphism rs1812594 of the GNRH 1 gene was observed in 4 patients (4.4%). According to the literature, the reported allele frequency of this SNP is 18–30% in Caucasians and 52–61% in Asians. One patient and one control subject were found to be heterozygous for SNP rs6186, which has an allele frequency of 1-4% [26].

**Table 4:** Average hormone values in the studied girls on the 14th day of the cycle.

| Hormones | Control n= 10 | Girls n= 17 | |
|---|---|---|---|
| STH | 3.9±0.2ng/ml | 1.3± 0.2 | p >0.05 |
| IGF-1 | 163.5±9.8 ng/ml | 112.8±10.2 | p <0.05 |
| LH | 13.2±2.7 mIU/L | 1.4 ±0.1 | p <0.05 |
| FSH | 10.4±2.5 mIU/L | 0.9±0.03 | p <0.05 |
| TSH | 2.7±0.7 mIU/L | 2.81±0.8 | p >0.05 |
| Prolactin | 5.8±0.3 ng/ml | 4.6 ±0.9 | P >0.05 |
| E2 | 129.1 ±10.6 pg/ml | 4.9±0.2 | P <0.05 |
| Cortisol | normal morning 596.5 ± 11.7 nmol/l | 595.25±13.3 | p >0.05 |
| free thyroxine | 16.5 ± 2.6 pmol/l | 14.8± 2.4 | p >0.05 |

Note: p - significance of differences compared to the control group (p <0.05). The table for comparison shows fluctuations in hormone levels from 11 to 16 years of age in the control group (healthy individuals); E2 – Estradiol.

**Table 5:** Clinical data of 10 patients examined with analysispolymorphismGNRH 1 gene (rs 6185, rs1812594).

| No. | Patient, clinical status | Diagnosis | karyotype | Deleted marker | Hormonal study | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Testosterone (n 0-38 nmol/l) | FSH (n 9.0-30.0 mIU/ml) | LH- (n 1.7-8.6 mIU/ml) | E2(normal 131-345 pmol/l |
| 1 | A., 14 years 3 months Growth SDS – 1.7 V testes 2 ml penis 2 sm G1 | nIGG | 46 XY | rs 6185 | 0.51 | 0.68 | 0.45 | - |
| 2 | Sh., 12 years 6 months Growth SDS + 1.0 V testes -abs penis 3 sm G1 | nIGG | 46XY | rs 6185 | 0.7 | 0.73 | 0.96 | - |
| 3 | R., 15 years old p 8.0 cm V tests -2 G1 | nIGG | 46XY | rs 6185 | 0.44 | 0.56 | 0.66 | - |
| 4 | S., 15 years old p – 8 cm V tests -3 G1 | nIGG | 46XY | rs 6185 | 0.087 | 0.44 | 0.46 | - |
| 5 | M. 14 years old SDS height -0.8 | nIGG | 46 XX | rs 6185 | 0.001 | 0.36 | 0.56 | 133 |
| 6 | K.14 years SDS growth -1.2 | nIGG | 46 XX | rs 6185 | 0.003 | 0.38 | 452 | 132 |
| 7 | S.14 years SDS growth -1.5 V tests -2 G1 | nIGG | 46XY | rs1812594 | 0.6 | 0.4 | 0.43 | - |
| 8 | R.14 years SDS growth -1.8 | nIGG | 46 XX | rs1812594 | 0.009 | 0.36 | 0.63 | 123 |
| 9 | M.14 years SDS growth -1.6 V tests -2 G1 | nIGG | 46XY | rs1812594 | 0.8 | 0.48 | 0.56 | - |
| 10 | S.14 years SDS growth -1.3 | nIGG | 46 XX | rs1812594 | 0.006 | 0.51 | 0.43 | 130 |

## Conclusion

1.      The most common patients among those examined were aged 14.7 years - 22 boys and 8 girls (IV stage according to Tanner by age). In this case, the stage of puberty upon examination corresponded to II both 22 boys (30.1%) and 8 girls (47%).

2.      In general, when assessing the stage of puberty, it was revealed that 30 adolescents had delayed puberty, that is, puberty corresponded to stage 2 at the age of 13-14 years in both boys and girls. At the same time, in group 1 of patients with nIH+ hypothyroidism, the lowest average values of height and weight were observed in comparison with group 1 of patients with nIHH.

3.      Our results confirm that gene polymorphism GNRH 1 is the genetic cause of nIHH. At the same time, out of 90 patients with clinical and hormonal data, nIHH was found in 5 (6.6%) cases. polymorphismrs 6185geneGNRH 1and in (4.4%) -rs1812594.

## References

1. Gore AC (2002) GnRH: The Master Molecule of Reproduction. Boston: Kluwer Academic Publishers.

2. Crowe S, Cushing H, Homans J (1910) Experimental hypophysectomy. Bull Johns Hopkins Hospital 21: 127-167.

3. Harris GW (1937) The induction of ovulation in the rabbit by electrical stimulation of the hypothalamo-hypophysial mechanism. Proc R Soc Lond B Biol Sci 122: 374-394.

4. Hinsey JC (1937) The relation of the nervous system to ovulation and other phenomena of the female reproductive tract.Cold Spring Harbor Symp Quant Biol 5: 269-279.

5. Brooks CM (1938) A study of the mechanism whereby coitus excites the ovulation-producing activity of the rabbit's pituitary. Am J Physiol 121: 157-177.

6. Taubenhaus M, Soskin S (1941) Release of luteinizing hormone from the anterior hypophysis by an acetylcholine-like substance from the hypothalamic region. Endocrinology 29: 958-968.

7. A V Schally, A Arimura, Y Baba, R M Nair, H Matsuo, et al. (1971) Isolation and properties of the FSH and LH-releasing hormone. Biochem Biophys Res Comm 43: 393-399.

8. M Amoss, R Burgus, R Blackwell, W Vale, R Fellows, et al. (1971) Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. Biochem Biophys Res Commun 44: 205-210.

9. Gajdos ZK, Hirschhorn JN, Palmert MR (2009) What controls the timing of puberty? An update on progress from genetic investigation. Curr Opin Endocrinol Diabetes Obes 16: 16-24.

10. Kim HG, Bhagavath B, Layman LC (2008) Clinical manifestations of impaired GnRH neuron development and function. Neurosignals 16: 165-182.

11. B Franco, S Guioli, A Pragliola, B Incerti, B Bardoni, et al. (1991) A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature 353: 529-536.

12. R Legouis, J P Hardelin, J Levilliers, J M Claverie, S Compain, et al. (1991) The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. Cell 67: 423-435.

13. Catherine Dodé, Jacqueline Levilliers, Jean-Michel Dupont, Anne De Paepe, Nathalie Le Dû, et al. (2003) Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet 33: 463-465.

14. John Falardeau, Wilson C J Chung, Andrew Beenken, Taneli Raivio, Lacey Plummer, et al. (2008) Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest 118: 2822-2831.

15. Catherine Dodé, Luis Teixeira, Jacqueline Levilliers, Corinne Fouveaut, Philippe Bouchard, et al. (2006) Kallmann syndrome: Mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet 2: e175.

16. Hyung-Goo Kim, Ingo Kurth, Fei Lan, Irene Meliciani, Wolfgang Wenzel, Soo Hyun Eom, et al. (2008) Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet 83: 511-519.

17. R S Jackson, J W Creemers, S Ohagi, M L Raffin-Sanson, L Sanders, et al. (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet 16: 303-306.

18. Nicolas de Roux, Emmanuelle Genin, Jean-Claude Carel, Fumihiko Matsuda, Jean-Louis Chaussain, et al. (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS-1-derived peptide receptor GPR54. Proc Natl Acad Sci USA 100: 10972-10976.

19. Stephanie B Seminara, Sophie Messager, Emmanouella E Chatzidaki, Rosemary R Thresher, James S Acierno Jr, et al. (2003) The GPR54 gene as a regulator of puberty. N Engl J Med 349: 1614-1627.

20. N De Roux, J Young, M Misrahi, R Genet, P Chanson, G Schaison, et al. (1997) A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med 337: 1597-1602.

21. A Kemal Topaloglu, Frank Reimann, Metin Guclu, Ayse Serap Yalin, L Damla Kotan, et al. (2008) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 41: 354-358.

22. Cattanach BM, Iddon CA, Charlton HM, Chiappa SA, Fink G (1977) Gonadotropin-releasing hormone deficiency in a mutant mouse with hypogonadism. Nature 269: 338-340.

23. A J Mason, J S Hayflick, R T Zoeller, W S Young, H S Phillips, et al. (1986) A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse. Science 234: 1366-1371.

24. A J Mason, S L Pitts, K Nikolics, E Szonyi, J N Wilcox, et al. (1986) The hypogonadal mouse: Reproductive functions restored by gene therapy. Science 234: 1372-1378.

25. Tiong J, Locastro T, Wray S (2007) Gonadotropin-releasing hormone-1 (GnRH-1) is involved in tooth maturation and biomineralization. Dev Dyn 236: 2980-2992.

26. Chan YM, De Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, et al. (2009) GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci US A 106(28): 11703-11708.

**Assets of Publishing with us**

- Global archiving of articles
- I*mm*ediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles

https://biomedres.us/