

HOMOZYGOUS P.R31H *GNRH1* MUTATION AND NORMOSMIC CONGENITAL HYPOGONADOTROPIC HYPOGONADISM IN A PATIENT AND SELF-LIMITED DELAYED PUBERTY IN HIS RELATIVES

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Abstract

Objectives: Congenital hypogonadotropic hypogonadism (CHH) is a rare condition resulting from GnRH deficiency. Gonadotropin Releasing Hormone 1 (*GNRH1*) homozygous mutations are an extremely rare cause of normosmic CHH (nCHH). Most heterozygous individuals are asymptomatic, with the notable exception of individuals heterozygous for a p.R31C *GNRH1* mutation.

Case presentation: The patient is an index case from a consanguineous family, presenting with severe CHH and his parents presenting with late puberty and normal fertility. The index case is homozygous for a p.R31H *GNRH1* variant, both parents being heterozygous. The analysis of a panel of genes implicated in CHH does not show any other clinically relevant variant in any other gene tested.

Conclusions: *GNRH1* mutations are a rare cause of nCHH. Five different mutations have been reported so far in homozygous individuals. Most are frameshift in nature but the one reported here causes an amino acid change in the Gonadotropin-releasing hormone (GnRH) decapeptide. Both independently reported patients with the p.R31H mutation are from Turkish origin. The question of the possible role of this mutation in the late puberty of the heterozygous parents needs further documentation. An analogy is made with the heterozygous individuals carrying the p.R31C and displaying partial CHH. No nonreproductive disorder is noted.

Introduction

Congenital hypogonadotropic hypogonadism (CHH (MIM: 146110)) is a rare condition resulting from GnRH deficiency. In its severe form, it clinically manifests in boys by micropenis and cryptorchidism and by absent pubertal development in both sexes. Sex steroids are low with low to normal

gonadotropin levels and an otherwise normal pituitary function. CHH is genetically heterogeneous; it is due to either a gonadotropic neuron migration defect during embryogenesis or a defect in the synthesis, secretion or action of Gonadotropin Releasing Hormone (GnRH). An oligogenic inheritance is documented in many cases and could explain the observed phenotypic variability. Because of the common embryologic development of the olfactory and the gonadotropic neurons, CHH can be accompanied by anosmia (Kallman syndrome) or be normosmic [1, 2].

Although Gonadotropin Releasing Hormone 1 (*GNRH1*) was the obvious candidate gene for normosmic CHH (nCHH), initial studies [2] did not find any *GNRH1* mutation within the CHH population. Because of their exceptional rarity, it took some 30 years for the first *GNRH1* mutation to be described [3, 4].

The low frequency of *GNRH1* mutations in CHH might in part result from the short length of the gene [5]. *GNRH1* encodes the preprohormone that is ultimately processed to produce the GnRH decapeptide. The decapeptide is secreted by the GnRH neurons in the hypothalamus and triggers an intracellular cascade leading to the synthesis and secretion of Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary gonadotrophs.

So far, seven independent patients harbouring homozygous *GNRH1* mutations have been reported (five different mutations), all with a severe nCHH phenotype. We report on an eighth homozygous patient with nCHH for a previously reported *GNRH1* mutation that leads to an amino acid change within the GnRH decapeptide.

Case report

The index case was evaluated at 8 months of age for cryptorchidism and microphallus. His parents are second cousins of Aramaic origin, from a Turkish village close to Syria. His mother had late menarche at 15.5 years and normal fertility with a first pregnancy at 17 years. His father had late puberty but had his first child at 21 years. The index case has three healthy siblings among which two sisters had late menarche (at 16 and 15.5 years). The patient's examination was notable for palpable inguinal testes, microphallus (2.8 x 0.9 cm), distal balanic hypospadias, unilateral single palmar crease and absence of midline or skeletal defects. Inhibin B was 89.4 ng/L. A GnRH stimulation test was performed. Baseline FSH and LH levels were 0.7 IU/L and <0.2 and rose to 6 and 1.2 IU/L, respectively. A human chorionic gonadotropin stimulation test produced a modest change in serum testosterone (from <1 to 29 ng/dl). Karyotype was 46 XY.

Ultrasound identified inguinal testes with dimensions of 8 x 5 x 5 and 8 x 5 x 4 x 6 mm and normal kidneys. At the age of 9 months, he was treated with 25 mg of testosterone enanthate every 4 weeks for three months. Penile length increased to 4 cm. Shortly thereafter, he underwent left orchiopexy and balanic hypospadias repair. Testicular epididymal dissociation was noted by the surgeon on the left side.

At 14.1 years, the patient was still prepubertal, with small testis volume (1 mL) and a penile length of 4 cm. The GnRH stimulation test was repeated. Baseline FSH and LH levels were 1.01 IU/L and 0.3 and rose to 2.34 and 2.68 IU/L, respectively, and inhibin B level was 20 ng/L. Anti-Müllerian hormone level was 28.2 µg/L. Smell testing with a set of odorants revealed a normal sense of smell. He began treatment with 50 mg of testosterone enanthate every 4 weeks, which resulted in linear growth and

development of secondary sexual characteristics but testicular volume remained very small (2 mL). Fasting glucose and insulin levels were normal (78 mg/dL and 58 pMol/L, respectively) as was lipid profile, although a tendency for low muscle mass and abdominal adiposity was noted with a Body Mass Index at 0.4 standard deviation score. His academic achievements were in the normal range, and no behavioural troubles were reported.

He is currently 15 years old and still receives testosterone replacement therapy.

Blood samples were obtained from the patient and his parents after provision of proper informed consent. DNA was extracted in accordance with standard procedures. Targeted clinical exome capture (4427 genes) was achieved in the index case using the SeqCap EZ Choice XL (NimbleGen, Madison, WI). The samples were subsequently sequenced in a paired-end 125-bp run on a HiSeq 1500 instrument (Illumina, San Diego, CA) at the Brussels Interuniversity Genomics High Throughput core (BRIGHTcore, Brussels, Belgium). A bioinformatic pipeline was launched by BRIGHTcore, and filtering of the variants was accomplished using Highlander software (available at: <http://sites.uclouvain.be/>). In silico analysis of a list of 167 genes involved in disorders of sex development was performed. Details on pipeline, filtering and gene lists are available on request. The analysis of the data revealed a homozygous c.92G>A, p.Arg31His variant in the exon 1 of the *GNRH1* gene (GCh37/hg19 chr8:25280755G>A, NM_000825) in the affected patient. Both parents were heterozygous for the same variant.

The parents of the index case have given their written informed consent to publish their case and the case of their child. The study was approved by the ethical committee of the Hôpital Universitaire des Enfants Reine Fabiola.

Discussion

We report on the eighth patient with nCHH due to a homozygous *GNRH1* mutation.

Homozygous *GNRH1* mutations are a very rare cause of nCHH, as illustrated by the seven reported cases out of more than 1,000 patients screened for the genetic aetiology of their nCHH (Table 1) [3, 4, 6, 7, 8–10] gnomAD (<https://gnomAD.broadinstitute.org>), without occurrence at the homozygous state. It has been reported at the homozygous state in a sibship of Turkish origin with CHH [8]. This variant affects the arginine in position 31 of the GnRH preprohormone which corresponds to the arginine in position 8 of the GnRH decapeptide, substituting it with a histidine.

The arginine in position 8 of the GnRH decapeptide has been shown to be crucial for biological activity [5].

All the reported patients homozygous for *GNRH1* mutations have a severe nCHH phenotype with micropenis, cryptorchidism in boys and absent pubertal development in both sexes. The phenotypic expression in heterozygous individuals remains an open question. In the literature, the pubertal timing of heterozygous siblings and parents of homozygous *GNRH1* patients is reported to be normal (Table 1). By contrast, we document pubertal delay with normal fertility in the heterozygous parents of our patient.

Table 1: Patients with nCHH homozygous for *GNRH1* mutations. All cases have a severe nCHH phenotype, and heterozygous family members are described as asymptomatic.

Authors	Patients with nCHH screened (n)	<i>GNRH1</i> genotype		Affected members of the same family (n)	Geographical origin
		cDNA	Amino acid		
Chan et al. 2009 [4]	310	c.87delA/c.87delA	p.G29GfsX12	1 male	Armenia
Cassatella et al. 2018 [9]	55	c.87delA/c.87delA	p.G29GfsX12	1	Europe
Mengen et al. 2016 [8]	50	c.87delA/c.87delA	p.G29GfsX12	1 male	Turkey
		c.92G>A/c.92G>A	p.R31H	1 female, 1 male	Turkey
Bouligand et al. 2009 [3]	-	c.18-19insA/c.18-19insA		1 female, 1 male	Romania
Amato et al. 2019 [10]	75	c.142-2A>C/c.142-2A>C	STOP codon	1 male	Brazil
Sagi et al. 2020 [12]	-	c.119_122dupGAGA/c.119_122dupGAGA	p.Asp37GlufsTer8	1 female, 1 male	Pakistan
Miraoui 2013 [7]	187	-	-	-	-
Maione 2013 [5]	410 (nCHH and KS)	-	-	-	-
This report		c.92G>A/c.92G>A	p.R31H*	1 male	Turkey

In italic, the index case and another patient reported with the same mutation.
 nCHH, normosmic congenital hypogonadotropic hypogonadism; KS, Kallman syndrome.

Of note, partial forms of nCHH have been described in patients heterozygous for a substitution of the same Arg³¹ into a cysteine in *GNRH1* (Table 2). Chan in 2009, Quaynor in 2011 and Maione in 2013 report on nine patients, from four families, heterozygous for a p.R31C *GNRH1* variant and displaying a milder phenotype of pubertal arrest in boys and amenorrhoea with subfertility in women [4, 5, 11].

Table 2: Patients with CHH heterozygous for the p.R31C mutation of *GNRH1*.

Authors	Number of affected members of the same family	Phenotype
Chan et al. 2009 [4]	4 females	Spontaneous reversal of amenorrhoea, ovarian stimulation to conceive.
Quaynor 2011 [11]	1	No phenotype provided
Maione 2013 [5]	Family 1: 1 male de novo Family 2: 1 male, 1 female	Pubertal arrest in men; amenorrhoea, ovarian stimulation to conceive in women

nCHH, normosmic congenital hypogonadotropic hypogonadism.

Both the p.R31C and p.R31H mutations cause an amino acid substitution localised in the position 8 of the GnRH decapeptide itself rather than a frameshift. Some phenotypic expression is observed in individuals heterozygous for the p.R31C and p.R31H mutations but not in individuals heterozygous for a frameshift *GNRH1* mutation.

In conclusion, *GNRH1* mutations are a rare cause of nCHH. Five different mutations have been reported so far in 8 index cases. Most are frameshift in nature but the one reported here causes an amino acid change in the GnRH decapeptide. Both independently reported patients with this mutation are from Turkish origin. No nonreproductive disorder was noted in the patient. The question of the possible role of this mutation in the late puberty of the heterozygous parents needs further documentation.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The parents of the index case have given their written informed consent to publish their case and the case of their child. The study was approved by the ethical committee of the Hôpital Universitaire des Enfants Reine Fabiola.

References

1. Maione L, Dwyer AA, Francou B, Guiochon-Mantel A, Binart N, Bouligand J, et al. Genetics in endocrinology: Genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: new challenges in the era of oligogenism and next-generation sequencing. *Eur J Endocrinol* 2018;178:R55-80.
2. Boehm U, Bouloux P-M, Dattani MT, de Roux N, Dodé C, Dunkel L, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 2015;11:547-64.
3. Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, et al. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med* 2009;360:2742-8.
4. Chan Y-M, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsirias S, et al. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A* 2009; 106:11703-8.
5. Maione L, Albarel F, Bouchard P, Gallant M, Flanagan CA, Bobe R, et al. R31C GNRH1 mutation and congenital hypogonadotropic hypogonadism. *PloS One* 2013;8:e69616.
6. Weiss J, Crowley WF, Jameson JL. Normal structure of the gonadotropin-releasing hormone (GnRH) gene in patients with GnRH deficiency and idiopathic hypogonadotropic hypogonadism*. *J Clin Endocrinol Metabol* 1989;69:299-303.
7. Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al. Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet* 2013;92:725-43.
8. Mengen E, Tunc S, Kotan LD, Nalbantoglu O, Demir K, Gurbuz F, et al. Complete idiopathic hypogonadotropic hypogonadism due to homozygous GNRH1 mutations in the mutational Hot Spots in the region encoding the decapeptide. *Horm Res Paediatr* 2016;85: 107-11.

9. Cassatella D, Howard SR, Acierno JS, Xu C, Papadakis GE, Santoni FA, et al. Congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty have distinct genetic architectures. *Eur J Endocrinol* 2018;178:377-88.
10. Amato LGL, Montenegro LR, Lerario AM, Jorge AAL, Guerra Junior G, Schnoll C, et al. New genetic findings in a large cohort of congenital hypogonadotropic hypogonadism. *Eur J Endocrinol* 2019;181:103-19.
11. Quaynor SD, Kim H - G, Cappello EM, Williams T, Chorich LP, Bick DP, et al. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertil Steril* 2011;96:1424-30.e6.
12. Sagi SV, Joshi H, Whiles E, Hikmat M, Puthi VR, MacDougall J, et al. Normosmic idiopathic hypogonadotropic hypogonadism due to a novel GNRH1 variant in two siblings. *Endocrinol Diabetes Metab Case Rep* 2020;2020. <https://doi.org/10.1530/EDM-19-0145>.