Role of gonadotropin-releasing hormone receptor mutations in patients with a wide spectrum of pubertal delay

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Objective: To analyze the GNRHR in patients with normosmic isolated hypogonadotropic hypogonadism (IHH) and constitutional delay of growth and puberty (CDGP).
Design: Molecular analysis and in vitro experiments correlated with phenotype.
Setting: Academic medical center.
Patient(s): A total of 110 individuals with normosmic IHH (74 male patients) and 50 with CDGP.
Intervention(s): *GNRHR* coding region was amplified and sequenced.
Main Outcome Measure(s): Novel variants were submitted to in vitro analysis. Frequency of mutations and genotype-phenotype correlation were analyzed. Microsatellite markers flanking *GNRHR* were examined in patients carrying the same mutation to investigate a possible founder effect.
Result(s): Eleven IHH patients (10%) carried biallelic *GNRHR* mutations. In vitro analysis of novel variants (p.Y283H and p.V134G) demonstrated complete inactivation. The founder effect study revealed that Brazilian patients carrying the p.R139H mutation shared the same haplotype. Phenotypic spectrum in patients with *GNRHR* mutations varied from complete GnRH deficiency to partial and reversible IHH, with a relatively good genotype-phenotype correlation. One boy with CDGP was heterozygous for the p.Q106R variant, which was not considered to be pathogenic.
Conclusion(s): *GNRHR* mutations are a frequent cause of congenital normosmic IHH and should be the first candidate gene for genetic screening in this condition, especially in autosomal recessive familial cases. The founder effect study suggested that the p.R139H mutation arises from a common ancestor in the Brazilian population. Finally, mutations in *GNRHR* do not appear to be involved in the pathogenesis of CDGP. (Fertil Steril® 2014;102:838–46. ©2014 by American Society for Reproductive Medicine.)
Key Words: GnRH receptor, hypogonadotropic hypogonadism, pubertal delay, mutation, founder effect

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Normal pubertal development and reproductive function depends on the intact release and action of hypothalamic gonadotropin-releasing hormone (GnRH), the fundamental regulator of the gonadotropic axis. The beginning of puberty is characterized by reactivation of pulsatile GnRH secretion, after a period of quiescence during childhood (1).

Delay in the normal timing of pubertal onset is the underlying feature of constitutional delay of growth and puberty (CDGP) (2). CDGP is considered to be an extreme of the normal spectrum in pubertal timing, with individuals spontaneously starting and attaining complete pubertal development 2.5 standard deviations later than the mean age (2). Although CDGP represents the most common single cause of delayed puberty within the general population, it can be diagnosed only after other underlying conditions have been ruled out. The main differential diagnosis of CDGP is congenital normosmic isolated hypogonadotropic hypogonadism (IHH). Congenital IHH is characterized by failure of gonadal function secondary to deficient GnRH-induced gonadotropin secretion (3). IHH is a clinically and genetically heterogeneous condition that can be sporadic or inherited as an autosomal trait (4). The phenotypic spectrum of patients with normosmic IHH varies from complete lack of secondary sexual characteristics and absent gonadotropin secretion to incomplete pubertal development (4). When IHH is associated with olfactory abnormalities, it characterizes Kallmann syndrome, associated with defects in the migration of olfactory and GnRH neurons during fetal life.

GnRH receptor (*GNRHR*)–inactivating mutations were the first recognized monogenic cause of normosmic IHH (5). Since the first description by de Roux et al. in 1997 (5), more than 20 mutations distributed throughout the coding sequence of the receptor have been reported in patients with sporadic or familial forms of normosmic IHH, in a classic autosomal recessive mode of inheritance (5–14). In addition, a number of other genes have been implicated in the pathogenesis of IHH (15).

In contrast to IHH, no specific genes have been identified in association with the molecular pathogenesis of CDGP to date. Nevertheless, genetic variation is known to influence the normal spectrum of pubertal timing. CDGP frequently aggregates in families, with a reported incidence of 50%–75% of patients with a family history of delayed puberty, suggesting that it may be at least in part genetically determined (2, 16). Mutations in genes underlying IHH have been reported in families with a wide phenotypic spectrum, varying from pubertal delay to severe IHH. CDGP may represent a mild phenotype of IHH, and defects in *GNRHR* could contribute to this phenotype.

In the present study, we examined the frequency and phenotype-genotype correlation of *GNRHR* mutations in a large cohort of patients with pubertal delay, including normosmic IHH and constitutional delay of growth and puberty. We also performed a founder effect study of a recurrent *GNRHR* mutation.

MATERIAL AND METHODS

Patients

The protocol was approved by the Ethical Committee of Hospital das Clinicas, Sao Paulo University. Written informed consents were obtained from all patients or their parents. A total of 160 patients with pubertal delay were studied: 50 with CGDP (41 [82%] boys and 9 [18%] girls) and 110 with normosmic IHH (74 [67.3%] male and 36 [32.7%] female). Twenty patients (18%) had familial IHH. *GNRHR* had been previously sequenced in 15 patients from this cohort (8). Other genes associated with the IHH phenotype, including *TAC3, TACR3, KISS1, KISS1R, GNRH1, FGF8, FGFR1, PROK2, PROKR2,* and *WDR11,* have been previously sequenced in the IHH group (17–24). The control population was composed of 150 healthy Brazilian adults of both sexes with normal pubertal development.

The diagnosis of CDGP was based on lack of breast development (Tanner stage 2) by the age of 13 years and absent menarche by the age of 15 years in girls, testicular volume $<$4.0 mL by the age of 14 years in boys, delayed bone age, absence of other identifiable causes of delayed puberty, and spontaneous and complete achievement of puberty development by age 18 years during follow-up. The diagnosis of normosmic IHH was based on the following criteria: incomplete or absent pubertal development after 18 years in males and 16 years in females, prepubertal or low testosterone (T) or estradiol (E_2) levels for age, low or normal basal gonadotropin levels, and otherwise normal pituitary function, normal hypothalamic-pituitary imaging, and normal olfaction confirmed by a formal olfactory test (Smell Identification Test or Alcohol Sniff Test) (25).

Hormone Assays

Serum LH and FSH concentrations were measured by radioimmunoassay up to 1991 or immunofluorometric assays (Autodelfia hLH Spec and Autodelfia hFSH) after 1991. The sensitivity of the latter was set at 0.1 IU/L for LH and 1.0 IU/L for FSH. Serum T and E_2 concentrations were measured by commercial solid-phase fluoroimmunoassay (Autodelfia Testosterone; PerkinElmer). The interassay coefficient of variation was $\leq$5% for all assays. For the acute GnRH stimulation test, serum LH and FSH were measured at −15, 0, 15, 30, 45, and 60 minutes after intravenous (IV) administration of 100 μg GnRH.

DNA Analysis

Genomic DNA was extracted from all patients, and the three exons of *GNRHR* (NM_000406.2) were amplified with the use of polymerase chain reaction (PCR) using specific intronic primer pairs (8). PCR products were purified and automatically sequenced in an ABI Prism Genetic Analyzer 3100 (Perkin-Elmer) (26).

In Silico Analysis

Mutation Taster (www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (Sorting Intolerant from Tolerant Human Protein) (http://sift.jcvi.org/www/SIFT_enst_submit.html) programs were used to predict the putative impact of an amino acid substitution on protein structure and function and to predict the pathogenicity of the missense variants (27).