

BRIEF REPORT

A Homozygous R262Q Mutation in the Gonadotropin-Releasing Hormone Receptor Presenting as Constitutional Delay of Growth and Puberty with Subsequent Borderline Oligospermia

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Context: The GnRH receptor plays a central role in regulating gonadotropin synthesis and release, and several mutations in the *GNRHR* gene have been reported in patients with idiopathic or familial forms of isolated hypogonadotropic hypogonadism (IHH).

Objective: The objective of the study was to investigate whether partial loss-of-function mutations in the GnRH receptor might be responsible for delayed puberty phenotypes.

Patients: Patients included sibling pairs with delayed puberty ($n = 8$) or those in whom one brother had delayed puberty and another had hypogonadotropic hypogonadism ($n = 3$).

Methods: Methods included mutational analysis of the *GNRHR* gene.

Results: A homozygous R262Q mutation in the GnRH receptor was identified in two brothers from one family. In this kindred, the

proband presented at 15 yr of age with delayed puberty. After a short course of testosterone, he seemed to be progressing through puberty appropriately and was discharged from follow-up. His younger brother was also referred with delayed puberty but showed little progress after treatment. Frequent sampling revealed detectable but apulsatile LH and FSH release. His clinical progress was consistent with IHH, and he requires ongoing testosterone replacement.

Conclusions: Homozygous partial loss-of-function mutations in the GnRH receptor, such as R262Q, can present with variable phenotypes including apparent delayed puberty. Ongoing clinical vigilance might be required when patients are discharged from follow-up, especially when there is a family history of delayed puberty or IHH because oligospermia and reduced bone mineralization can occur with time. (*J Clin Endocrinol Metab* 91: 5117–5121, 2006)

CONSTITUTIONAL DELAY OF growth and puberty (CDGP) is one of the most frequent reasons for referral to the pediatric/adolescent endocrinology clinic. In most situations puberty will occur normally, albeit at a later age, but in a small proportion of cases, delayed puberty may reflect a more significant underlying endocrine condition, such as hypogonadotropic hypogonadism or panhypopituitarism (1).

Differentiating these rare but serious conditions from more prevalent and benign conditions such as CDGP can be difficult. A short course of low-dose sex steroids is often useful to induce early secondary sexual characteristics and to stimulate endogenous puberty. A diagnosis of CDGP can usually be made if puberty continues to progress normally after this treatment, and the young adult can be discharged

from further follow-up and avoid unnecessary medicalization. However, failure to progress naturally in puberty, or to obtain an appropriate growth spurt, may suggest that further investigations or full pubertal induction are necessary.

In the past decade, several monogenic causes of hypogonadotropic hypogonadism have been identified. Some of these conditions have associated features such as hyposmia or abnormal neuronal migration (*KAL*, *FGFR1*, *NELF*), obesity (leptin, leptin receptor, *PC1*), hypopituitarism (*HESX1*, *LHX3*, *SOX3*, *PROP1*), or adrenal insufficiency (*DAX1*) (1, 2). Isolated hypogonadotropic hypogonadism (IHH) has been reported due to mutations in the genes encoding the GnRH receptor (*GNRHR*) or *GPR54* (3–6). We hypothesized that milder loss-of-function mutations in these genes could present as delayed puberty and chose to address this by studying the *GNRHR* gene in families in whom different siblings had either IHH or pubertal delay.

Subjects and Methods

Subjects

Samples were obtained from eight families with delayed puberty (no pubertal development by 14 yr in males) and three families in which

First Published Online September 12, 2006

Abbreviations: A, Axillary hair; CDGP, constitutional delay of growth and puberty; CV, coefficient of variation; G, genital stage; *GNRHR*, GnRH receptor; IHH, isolated hypogonadotropic hypogonadism; P, pubic hair.

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different siblings had IHH or delayed puberty. No obvious associated features suggestive of a more complex syndromic cause of hypogonadotropic hypogonadism (such as anosmia, obesity, pituitary dysfunction, or adrenal failure) were present.

Mutational analysis

After obtaining institutional review board approval and informed consent, DNA was extracted from patients' blood leukocytes using standard methods. The three coding exons and splice sites of the *GNRHR* gene were PCR amplified using primer pairs and conditions described previously (3). Sequencing reactions were performed using a BigDye terminator cycle sequencing kit (version 1.1; Applied Biosystems, Foster City, CA) and MegaBACE1000 DNA sequence analyzer (Amersham Biosciences, Buckinghamshire, UK) with Sequence Analyzer (version 3.0; Amersham Biosciences) and Sequencher (version 4.1; Genecodes Corp., Ann Arbor, MI) software.

Hormone assays

Serum LH concentrations were measured using an immunoradiometric assay (North-east Thames Regional Immunoassay Services, St. Bartholomew's Hospital, London, UK). The within-assay coefficients of variation (CVs) were 5.6, 3.6, 5.2, and 3.0% at serum LH concentrations of 2.9, 7.9, 18.3 and 35.8 IU/liter and the between-assay CV values were 10.4, 3.1, and 5.4% at serum concentrations of 4.7, 34.0, and 51.7 IU/liter, respectively. The sensitivity of the assay was 0.5 IU/liter. Serum FSH concentrations were measured by immunoradiometric assay (North-east Thames Regional Immunoassay Services). The within-assay CV values were 10.7, 7.6, 7.8, and 4.3% at serum concentrations of 2.8, 5.8, 13.2, and 26.1 IU/liter and the between-assay CV values were 8.1, 4.9, and 5.1% at serum concentrations of 2.9, 4.9, and 5.1 IU/liter, respectively. The sensitivity was 0.5 IU/liter.

Gonadotropin profiling and pulse analysis

Gonadotropin profiles were performed by withdrawing blood samples from an indwelling iv catheter every 20 min for a 24-h period. Gonadotropin pulsatility was analyzed by autocorrelation and Fourier transform (spectral power) using Time Series Analysis software (Easy TSA; Oxford University, Oxford, UK) with and without stationarization (differential and mean detrending) of the data (7).

Pubertal assessment

Pubertal rating was performed using standard ratings of Marshall and Tanner [genital stages 1–5 (G); pubic hair (P) stages 1–5; axillary hair (A) stages 1–3] (8).

Results

A *GNRHR* gene mutation was identified in one of the 11 families studied, in which one brother had been diagnosed with pubertal delay and another had IHH.

Case histories

The proband (I) was referred at 15.9 yr of age because of delayed puberty and poor growth (Fig. 1A). He was normosmic and had no associated features of note, and past medical and family history were unremarkable. The family was of Asian Indian ancestry. His birth weight was 2.2 kg. After a short course of oxandrolone, he was given 6 months treatment with testosterone undecanoate (Sustanon, 100 mg monthly) and reached pubertal ratings of G4, P4, A1, with 6- and 8-cc testes by 17.1 yr of age. Puberty continued to progress spontaneously. By 17.9 yr of age, he had ratings of G5, P5, A2, with 12- and 15-cc testes and was discharged from further regular follow-up.

The younger brother (II) was referred at 11.7 yr with con-

cerns about short stature (Fig. 1B). His birth weight was 2.7 kg and he had mild asthma. He was prepubertal and received a course of oxandrolone as a growth-promoting agent. He remained prepubertal at 14.3 yr of age and was given a short course of testosterone undecanoate. However, at 15.5 yr of age, he showed no spontaneous pubertal development (G3, P3, A1, 2-cc testes bilaterally), so further investigations were performed.

A 24-h gonadotropin profile (samples every 20 min) was undertaken. Although spontaneous FSH and LH release was detected throughout the day and night (Fig. 2A), time series analysis with and without detrending showed no autocorrelation (data not shown) and a poorly defined rapid Fourier transform of low spectral power (Fig. 2B) (9). Spontaneous testosterone release was virtually undetectable (Fig. 2A), but prolonged administration of human chorionic gonadotropin (1500 IU twice weekly) for 1 month at 16.2 yr produced a significant testosterone response [687 ng/dl (23.7 nmol/liter)] (Fig. 1D), consistent with central hypogonadism. Spontaneous GH release was consistent with his age and pubertal stage, and thyroid function, prolactin, and a cortisol day curve (27.4 μ g/dl at 0800 h) were normal. A hypothalamic-pituitary magnetic resonance imaging scan and skeletal survey were unremarkable. Puberty was induced with increasing doses of testosterone. Reevaluation off all treatment at 18.5 yr showed a persistently low testosterone despite gonadotropin responses to LHRH stimulation (Fig. 1D). He was therefore continued on testosterone replacement therapy.

Both individuals were reassessed clinically in adulthood. The proband (I) was shaving but felt he had somewhat reduced libido. He had detectable gonadotropins and testosterone toward the lower end of the normal range (Fig. 1C). At 32 yr of age, he had reduced bone mineral density [t-scores, -2.0 (hip) and -3.7 (lumbar spine)]. Semen analysis showed borderline oligospermia (18 million sperm/ml, 67% abnormal forms, 60% motility). He has recently fathered a child.

The younger brother (II) had subnormal testosterone [191 ng/dl (6.6 nmol/liter)] 1 yr after electing to stop testosterone replacement (Fig. 1D). This finding may reflect delayed partial activation of his hypothalamic-pituitary-gonadal axis or possible up-regulation of hypothalamic-pituitary-gonadal activity after prior testosterone exposure. His bone mineral density was reduced [t-scores, -1.2 (hip) and -2.5 (lumbar spine)]. He continues on testosterone replacement but has not had semen analysis performed yet.

Mutational analysis

Direct sequencing revealed a homozygous R262Q (CGG→CAG) mutation in both brothers. The affected arginine is a highly conserved residue in the third intracellular loop of the GnRH receptor (Fig. 2C) (10). This change has been reported previously in a compound heterozygous state (including Q106R/R262Q, A129D/R262Q, and R262Q/Y284C) in several patients with IHH (3, 4, 11–15).

Discussion

Mutations in the *GNRHR* gene were first described in 1997, and to date, approximately 20 different homozygous or com-

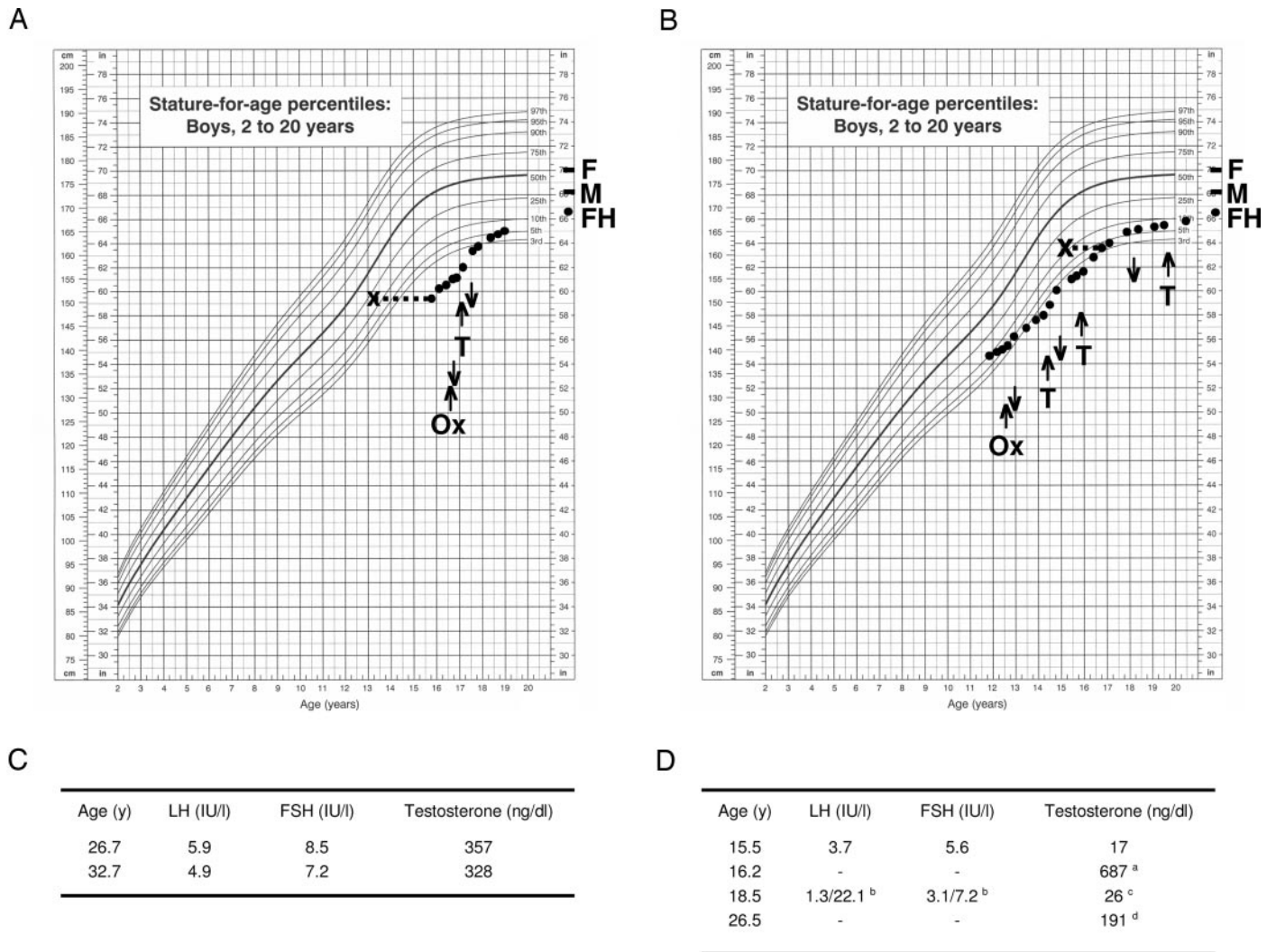


FIG. 1. Growth charts and response to treatment for both brothers. A, The proband (I) was diagnosed with constitutional delay of growth and puberty. B, The younger brother (II) was diagnosed with IHH. M, Maternal height; F, paternal height; FH, final height; Ox, oxandrolone; T, testosterone undecanoate. Upward arrows indicate start of treatment and downward arrows indicate treatment discontinuation. C, Endocrine data for the proband (I) showed testosterone values toward the lower part of the normal range (range 287–810 ng/dl; divide by 29 for nanomoles per liter). D, Endocrine data for the younger brother at times when off testosterone treatment; ^a, after human chorionic gonadotropin 1500 IU twice weekly for 1 month instead of testosterone; ^b, peak after LHRH stimulation; ^c, off testosterone 3 months; ^d, off testosterone 1 yr. Growth charts reproduced courtesy of the National Center for Health Science.

pound heterozygous changes have been reported in patients with idiopathic or familial forms of IHH (3, 4, 10–15). The two most frequent GNRHR mutations are Q106R and R262Q, accounting for approximately half of all reported changes (Fig. 2C) (16). Both these mutations usually occur in a compound heterozygous state and have been shown to cause partial loss of function in *in vitro* assays of receptor function (17).

The Q106R mutation causes decreased GnRH binding and possibly misfolding (3, 17, 18). Homozygous Q106R changes have been reported in association with milder phenotypes, such as partial IHH in a woman and the fertile eunuch syndrome in two males (14, 19, 20). These men had impaired secondary virilization and 15- to 17-cc testes. However, to our knowledge, spontaneous spermatogenesis associated with GNRHR mutations is rare (3).

The R262Q mutation described here has been reported in

a compound heterozygous state in patients of French Caucasian, English-American, and Irish-American ancestry with variable forms of IHH (3, 4, 11–15). Our finding of a homozygous R262Q mutation in an Asian Indian family suggests that the R262Q change represents a mutational hot spot rather than a founder effect. The functional consequences of the R262Q GNRHR mutation have been studied previously (3, 4, 17, 18). In general, this mutation does not affect GnRH binding or trafficking of the receptor to the gonadotrope cell surface but predominantly interferes with downstream signaling through several intracellular pathways [e.g. protein kinase C/inositol 1,4,5-triphosphate, ERK-1 (Gq/11), and cAMP (Gs)] (Fig. 2C) (3, 4, 10, 17).

Intracellular signaling by the GNRHR is complex and remains poorly understood because this single receptor must respond to pulsatile hypothalamic GnRH stimulation to regulate the synthesis and release of both FSH and LH as well

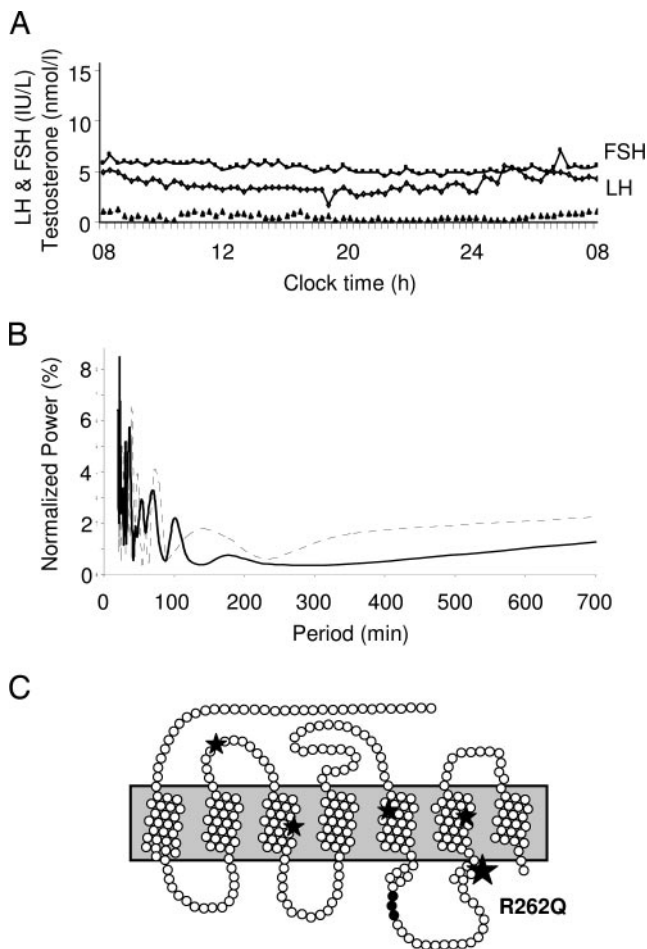


FIG. 2. A, Profile of spontaneous FSH (international units per liter) (■), LH (international units per liter) (◇), and testosterone (T, nanomoles per liter) (▲) release (samples every 20 min) over 24 h in the younger brother with IHH at 15.5 yr of age (testosterone, nanomoles per liter \times 29 for nanograms per deciliter). B, Time series analysis of FSH (solid line) and LH (broken line) revealed a poorly defined rapid Fourier transform of low spectral power. In this analysis, data are first stationary and log transformed to remove long-term trends, and then Fourier transformation is performed to deconvolute the complex signals into cosine and sine components, which separate and measure the different frequency components of the time series data array. The spectral power is an expression of the relative intensity of the signals at each frequency. A normal gonadotropin time series is likely to show dominant pulsatility of 120 min, with spectral power in the order of 20%. C, Pseudostructural plot of the GNRHR showing the position of the highly conserved arginine at position 262 within the third intracellular loop of the receptor. The R262Q change has been described previously in a compound heterozygous state in patients with IHH together with other disruptive mutations in the receptor (Q106R/R262Q, A129D/R262Q, and R262Q/Y284C). This amino acid is believed to form part of a critical interface with intracellular signaling pathways.

as likely having a tropic effect on the gonadotrope cell population. The presence of detectable but largely nonpulsatile gonadotropin release in the younger brother with IHH studied here shows that the R262Q mutation has a more detrimental effect on pulsatile gonadotropin release than gonadotropin synthesis. Furthermore, significant amounts of LH were released (22 IU/liter) after bolus GnRH stimulation, consistent with a greater effect of the R262Q mutation on FSH

regulation than LH (17). Taken together with functional studies, these findings suggest that the R262Q mutation produces a partial block in GNRHR signal transduction and reconfirms the importance of pulsatile gonadotropin signaling for normal pubertal development and reproductive function to occur. This kindred also highlights the phenotypic variability that can be seen in families with GNRHR mutations. It is likely that modifier genes that influence reproductive development and function could result in the variable phenotypic expression between these two brothers.

From the clinical perspective, this report shows that subtle phenotypes such as apparent constitutional delay of growth and puberty and borderline oligospermia can rarely occur due to partial loss-of-function mutations in the GNRHR in males. Obviously it would be inappropriate to recommend long-term follow-up for all young adults with delayed puberty because this is such a common condition, and it is important not to medicalize or stigmatize what might be considered a normal variant of pubertal timing. However, emphasis should be placed on individuals seeking endocrinological review if they have concerns regarding potency and fertility in the future, especially if there is an emerging family history of IHH. Detecting those young adults with partial forms of hypogonadotropic hypogonadism could have implications for long-term bone health, fertility, and psychosexual functioning, and defining the exact molecular basis is important for focusing treatment appropriately.

Acknowledgments

We are grateful to Jane Pringle and Nicola Bridges for their contribution to gonadotropin profiles and assays and Charles Brook and Richard Bell for their clinical support. We thank Stephanie Seminara, Phillippe Caron, and Nicholas de Roux for providing information about their patients' ethnic backgrounds. Research at the Institute of Child Health and Great Ormond Street Hospital for Children National Health Service Trust benefits from R&D funding received from the National Health Service Executive.

Received April 13, 2006. Accepted September 6, 2006.

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This work was supported by the Child Health Research Appeal Trust and The Wellcome Trust. J.C.A. holds a Wellcome Trust Clinician Scientist Fellowship (068061).

Disclosure statement: the authors have nothing to disclose.

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