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Hypogonadotropic hypogonadism due to GnRH receptor mutation in a sibling

Hipogonadyzm hipogonadotropowy u rodzeństwa z mutacją w genie receptora dla GnRH

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Abstract

Hypogonadotropic hypogonadism (HH) is characterised by delayed puberty and infertility. Congenital HH comprises Kallmann syndrome with hypo-/anosmia and idiopathic HH (IHH). The genetic origin remains unknown in most cases, but the defective GnRH receptor gene (GNRHR) accounts for a considerable proportion of IHH.

Here we describe a pair of siblings diagnosed with IHH. Aged 17 years, the boy was referred because of short stature (162 cm) and overweight (62.5 kg). He presented no signs of puberty, bone age of 14.5 years and insulin resistance. His sister, aged 16 years, also displayed delayed puberty. She was 166 cm tall and weighed 52 kg; her bone age was 12.5 years. Pelvic ultrasonography showed an infantile uterus and fibrous ovaries. In both siblings, serum gonadotropins were extremely low, and non-responsive to GnRH. Testosterone (1.38 nmol/l) and IGF1 (273 ng/ml) were decreased in the boy, although the girl did not present IFG1 deficiency. Her serum oestradiol was 10 pg/ml. MRIs of the hypothalamo-pituitary region and olfactory bulbs revealed them to be normal. The patients' sense of smell was unaltered. Their parents appeared to be first degree cousins. Considering the clinical data and potentially autosomal recessive HH transmission, the *GNRHR* gene was screened. The siblings turned out to be homozygous for the G416A transition, which had previously been identified in other HH individuals. The parents were heterozygous mutation carriers. The proband, moderately responding to LH, was started on low dose testosterone replacement, and his sister on transdermal oestradiol. Molecular data indicative of GnRH resistance could guide their future therapy should they desire fertility restoration. Further observations of the male patient may provide insights into androgen's influence on body mass, growth and insulin sensitivity.

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Key words: *GnRH receptor gene mutation, idiopathic hypogonadotropic hypogonadism, gender impact on IHH clinical features*

Streszczenie

Hipogonadyzm hipogonadotropowy (HH) charakteryzuje się opóźnionym dojrzewaniem i bezpłodnością. Wrodzony HH obejmuje zespół Kallamana z hipo-/anosmią oraz przypadki idiopatyczne (IHH). Podłoże genetyczne choroby pozostaje zazwyczaj niejasne, choć defekty genu receptora dla GnRH (GNRHR) odpowiadają za istotną część przypadków.

W pracy zaprezentowano opis pary rodzeństwa z rozpoznanym IHH. W wieku 17 lat u chłopca przeprowadzono konsultację z powodu niskorosłości (162 cm) i nadwagi (62,5 kg). Badania wykazały brak dojrzewania płciowego, wiek kostny oceniono na 14,5 roku oraz stwierdzono insulinooporność. U 16-letniej siostry również występowało opóźnione dojrzewanie. Chora miała 166 cm wzrostu, ważyła 52 kg; wiek kostny oceniono na 12,5 roku. Badanie ultrasonograficzne wykazało obecność dziecięcej macicy i włóknistych struktur jajników. U obojga rodzeństwa stwierdzono niskie stężenia gonadotropin w surowicy, bez reakcji na GnRH. Stężenie testosteronu (1,38 nmol/l) oraz IGF1 (273 ng/ml) były obniżone u chłopca, podczas gdy u dziewczynki nie wykazano niedoboru IGF1. Stężenie estradiolu w jej surowicy wynosiło 10 pg/ml. U obojga rezonans magnetyczny nie wykazał patologii okolicy podwzgórzowo-przysadkowej ani opuszek węchowych. Poczucie węchu nie było zaburzone.

Rodzice dzieci okazali się kuzynostwem pierwszego stopnia. Biorąc pod uwagę dane kliniczne oraz prawdopodobne autosomalne recesywne dziedziczenie HH, do badań molekularnych wybrano gen GNRHR. U rodzeństwa wykryto homozygotyczną tranzycję G416A, wcześniej opisywaną u innych chorych. U rodziców wykazano heterozygotyczne nosicielstwo mutacji. U probanda, wobec słabej reakcji na LH, włączono substytucję niskimi dawkami testosteronu, a u jego siostry — przezskórny preparat estradiolu. Z uwagi na oporność na GnRH wyniki badań molekularnych mogą wspomóc przyszłe decyzje terapeutyczne w przypadku prób indukcji gametogenezy. Dalsza obserwacja chłopca może dostarczyć danych na temat wpływu androgenów na skład masy ciała, wzrastanie oraz insulinowrażliwość.

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Słowa kluczowe: *mutacja genu receptora GnRH, idiopatyczny hypogonadotropowy hypogonadyzm, wpływ płci na kliniczny obraz IHH*

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Introduction

Hypogonadotropic hypogonadism (HH) is characterised by delayed or absent puberty and infertility. Gonads, deficient in gonadotropic stimulation, fail to undertake their hormonal and gametogenic function. HH may be due to acquired local lesions (tumour, trauma, infiltrative disorders, etc.) but can also arise from genetic defects that impair the development and/or function of the hypothalamo-pituitary region [1].

Congenital HH is a heterogenous condition, traditionally subdivided into Kallmannsyndrome associated with hypo-/anosmia, and idiopathic HH (IHH) which is diagnosed in patients without olfactory deficit [2].

Mutations in several genes have been identified in HH, although in the majority of cases the genetic background remains unknown. The genes responsible for Kallmann syndrome comprise *KAL1*, *FGF8*, *FGFR1*, *PROK2* and *PROKR2*, all involved in GnRH neurons migration during ontogenesis [3]. On the other hand, mutations leading to IHH have mostly been found in genes which are critical from the functional point of view [2, 4]. Precise pulsatile GnRH secretion is required for gonadal steroidogenesis and gametogenic function. Despite several attempts, it was only recently that a unique loss-of-function mutation (1-bp insertion 18insA) in the gonadotropin-releasing hormone gene (*GNRH1*) was found in a Romanian sibling with IHH [5]. Considering the rarity of GN*RH1* mutations, defective GnRH receptor was another logical candidate for IHH [6]. In fact, *GNRHR* mutations may account for up to 40% of familial, and 16.7% of sporadic, cases of normosmic HH [7]. Other genes recently associated with IHH include *GPR54/KISS1R*, *TAC3* and *TACR3*, which may all affect GnRH secretion [3, 4].

Case report

Here we describe a pair of siblings diagnosed with isolated hypogonadotropic hypogonadism. A boy and a girl were born at term to a consanguineous couple, following two uncomplicated pregnancies. They reached all motor milestones at the correct time, and their intellectual development was normal. A gradual decrease in growth velocity and increasing body mass were observed in the boy, starting approximately at the age of 11. Aged 17, he was referred to the endocrine department because of shortness (162 cm; < 3rd percentile) and overweight (62.5 kg, 25–50th percentile, BMI = 23.8 kg/m^2, Cole Index = 112%). Physical examination revealed no signs of pubertal development, a prepubertal penile size and small intrascrotal testes (1.5 ml each) without pathology on ultrasound. The patient's bone age was 14.5 years — his adjusted height was at the 25th percentile while Cole Index reached 121%. Serum gonadotropins were extremely low and did not respond to GnRH stimulation (Table I). Serum testosterone level was decreased (1.38 nmol/l) while TSH, free thyroxin, prolactin, ACTH, cortisol and DHEA-S were all within their normal ranges. IGF1 in his serum (273 ng/ml) was at the 20th percentile for chronological and bone age. His sense of smell was unaltered. MRI evaluation of the hypothalamo-pituitary region did not reveal any structural pathology and no abnormalities of the olfactory bulbs were noticed. An oral glucose tolerance test (OGTT) was performed, indicating impaired glucose tolerance (glucose rise from 93 to 173 mg/dl) with concomitant insulin resistance (insulin increase from 21.0 to 258.3 μIU/ml) [8].

Diagnosed with IHH, the patient was prescribed a 12-week course of hCG (Pregnyl 1500 IU i.m. thrice per week) and responded with a testosterone rise up to 5.86 nmol/l. One month after Pregnyl cessation, his testosterone was found to be low again (2.27 nmo/l), IGF1 was 309 ng/ml and Tanner's stage had not progressed except for the mild stage 2 of pubarche. Substitution therapy with a mixture of testosterone esters (Omnadren 250) was introduced, starting at 50 mg i.m. every four weeks. A balanced, calorie-restricted diet and regular physical exercise were advised in order to decrease his body mass and improve insulin sensitivity.

Additionally, it turned out that his younger sister, aged 16, also displayed delayed puberty (lack of menarche, thelarche 1, axillarche 2, pubarche 1 Tanner's

Table I. ***Results of the GnRH stimulation test in the affected sibling***

	0′	30′	60′	90′
Male patient (17-years-old)				
LH [mIU/ml]	0	0	0	0
FSH [mIU/ml]	0.4	0.4	0.5	0.3
Female patient (16-years-old)				
LH [mIU/ml]	0.1	0.1	0.3	0.3
FSH [mIU/ml]	0.4	0.4	0.4	0.3

stage). The girl was 166 cm tall (50–75th percentile) and weighed 52 kg (25–50th percentile). Her bone age was 12.5 years. Pelvic sonography revealed an infantile uterus (1.5 cm length with 1mm endometrial layer) and two fibrous ovaries, without visible follicles. The girl did not present any olfactory deficit, nor any pathologies on her head MRI. Similarly to her brother, serum LH and FSH concentrations were extremely low, and non-reactive to exogenous GnRH (Table I). Serum oestradiol was decreased (10 pg/ml); testosterone and androstenedione were 0.65 nmol/l and 1.3 ng/ml, respectively. TSH, free thyroxin, prolactin, ACTH, cortisol and DHEA-S were all within their reference ranges. Her serum IGF1 (593 ng/ml) was at the 80–95th percentile for both chronological and bone age. The girl did not display disorders in glucose-insulin homeostasis as assessed by OGTT. Low dose (25–37.5 mcg/24 h) transdermal oestradiol substitution was introduced. Progress in breast development and vaginal mucus discharge were noted two months later.

The healthy parents of the affected adolescents appeared to be first degree cousins. This finding raised a suspicion of autosomal recessive mode of inheritance of HH. The parents and children consented to further molecular analyses. Genomic DNA was extracted from peripheral blood cells. Considering the clinical data, i.e. normosmic HH with no other congenital defects and potential autosomal recessive transmission, priority was given to the gene encoding GnRH receptor (*GNRHR*). All three exons were amplified by polymerase chain reactions with three sets of intronic primers (details available upon request). Products were purified and sequenced bi-directionally using BigDye terminator cycle sequencing ready reaction kit on an ABI PRISM automatic sequencer. Sequences obtained were aligned with the Genebank reference NC_000004.11. The siblings were found to be homozygous for the G>A transition at position 416 within exon 1 of the *GNRHR* gene (Figure 1). This nucleotide change results in substitution of arginine for histidine at codon 139 (R139H) within the conserved DRS amino acid motif. Molecular screening of the parents revealed that both of them were heterozygous carriers of the same mutation, which confirmed the suspected mode of inheritance of the disease.

Discussion

The human *GNRHR* gene is located on chromosome 4q21.2 and encodes a 328-amino acid membrane protein [9]. To date, 27 *GNRHR* mutations, which confer complete or partial GnRH resistance, have been found in patients with IHH. Various mutations may affect the receptor expression, ligand binding affinity or signal transduction, and therefore account for the different degrees of HH observed among patients [10]. The homozygous R139H *GNRHR* mutation was previously identified in a Brazilian female who presented with primary amenorrhea, lack of breast development at 18 years, and low serum gonadotropins, unresponsive to exogenous GnRH [11]. An unrelated male of Polish origin with a homozygous R139H mutation was subsequently described [12]. Aged 19, he lacked any features of puberty and functional assessment of his hypothalamo-pituitary-gonadal axis confirmed complete HH [12]. The cardinal role of the highly conserved arginine residue was further supported by the finding of another substitution at this site, R139C, in two Turkish sisters with IHH [13].

In vitro studies revealed that cells transfected with mutant 139H *GNRHR* cDNA displayed normal expression and membrane localisation of GnRH receptors. However, they were unable to respond properly to GnRH, apparently due to abolished ligand binding and subsequent lack of intracellular signal transduction [11]. The GnRH receptor belongs to the family of the rhodopsin-like G protein-coupled receptors, composed of seven transmembrane domains and an extracellular amino-terminus [9]. Its activation results in increased

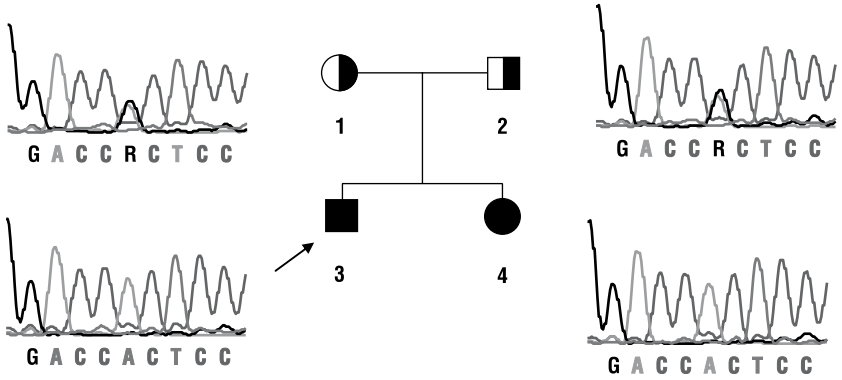

Figure 1. *Results of the GNRHR gene sequencing in the studied family*
Rycina 1. *Wyniki sekwencjonowania genu GNRHR u członków badanej rodziny*

activity of the phospholipase C and mobilisation of intracellular calcium stores [14]. The R139H mutation occurs within the conserved DRS motif at the junction of the third transmembrane domain and the second intracellular loop. It seems that protonation of the R139 residue, and its interaction with the neighbouring D138, are critical in maintaining active receptor conformation [15].

The results of mutational analysis did not substantially influence sex steroid replacement, which was instituted in both adolescents in order to achieve and maintain mature secondary sexual characteristics [16]. Nonetheless, it may provide useful hints if fertility restoration is desired. Due to the recessive inheritance pattern, patients with *GNRHR* mutations are unlikely to transmit the disease to their offspring, therefore fertility issues become of major concern. Most studies comparing pulsatile GnRH and hCG/hMG protocols in inducing gametogenesis among HH patients have revealed both approaches to be similarly effective [17]. However, in various cohorts, there are always individuals who fail to respond to this form of therapy. Molecular analysis might provide an explanation as to why some of these patients could be carriers of *GNRHR* mutations, resulting in complete GnRH resistance. On the other hand, cases of successful conception after high-dose GnRH pulsatile therapy have also been reported in subjects with milder receptor impairment, although pregnancy maintenance required repeated GnRH support [18]. Overall, gonadotropin stimulation seems more likely to induce ovulation/spermatogenesis in patients with *GNRHR* defects [18, 19].

By contrast, the phenotypic differences found in the studied sibling require further investigation. Siblings partially share a genetic background as well as most exogenous factors of the environment where they are brought up. Moreover, it is generally recognised that hypogonadism promotes increased body fat mass and insulin resistance in both genders. However, only the boy presented short stature and overweight complicated by insulin resistance. Impaired linear growth may also result from lack of pubertal androgens [20]. In healthy boys, the peak rise in testosterone levels (at G3/4 Tanner stage) coincides with an increase in mean 24 h hGH serum concentration and correlates with the pubertal growth spurt [21]. According to several studies, testosterone replacement therapy tends to improve insulin sensitivity and ameliorate body mass composition in males with IHH [22]. These parameters will require careful evaluation during follow-up of our patient.

Conclusion

Molecular analysis has enabled us to elucidate the aetiology of IHH in the studied siblings and, to some extent, may inform their future fertility treatment. Further observation of the male sibling may provide interesting insights into androgen's influence on body stature and insulin sensitivity in adolescents.

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