ORIGINAL RESEARCH

FREQUENCY OF THE ALLELIC VARIANT (Trp8Arg/Ile15Thr) OF THE LUTEINIZING HORMONE GENE IN A BRAZILIAN COHORT OF HEALTHY SUBJECTS AND IN PATIENTS WITH HYPOGONADOTROPIC HYPOGONADISM

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Berger K, Billerbeck AEC, Costa EMF, Carvalho LS, Arnhold IJP, Mendonca BB. Frequency of the allelic variant (Trp8Arg/Ile15Thr) of the luteinizing hormone gene in a Brazilian cohort of healthy subjects and in patients with hypogonadotropic hypogonadism. Clinics. 2005;60(6):461-4.

PURPOSE: To evaluate the frequency of allelic variant Trp8Arg/Ile15Thr in the luteinizing hormone β-subunit gene in a Brazilian population of healthy subjects and in patients with hypogonadotropic hypogonadism.

SUBJECTS AND METHODS: Two hundred and two adults (115 women) with normal sexual function and 48 patients (24 women) with hypogonadotropic hypogonadism underwent a molecular study of the the luteinizing hormone β-subunit gene using a polymerase chain reaction technique followed by enzymatic digestion with the restriction enzymes *Nco I* (for detection of the Trp8Arg point mutation) and *Fok I* (for detection of the Ile15Thr point mutation). Basal luteinizing hormone and FSH, testosterone, or estradiol levels were measured in 37 healthy subjects (21 women) and in 27 hypogonadotropic hypogonadism patients (13 women) by immunofluorometric methods (hLH-Spec and hFSH-Spec, AutoDELFIA, Wallac Oy, Turku, Finland).

RESULTS: The genetic variant of the luteinizing hormone β-subunit gene was present at a similar frequency in healthy subjects (14.4%) compared to patients with hypogonadotropic hypogonadism (16.6%). There was no effect of the allelic variant of the luteinizing hormone β-subunit gene on luteinizing hormone levels in patients with hypogonadotropic hypogonadism as compared to healthy subjects.

CONCLUSION: This study indicates that the allelic variant Trp8Arg/Ile15Thr of the luteinizing hormone β-subunit gene is a common polymorphism in the Brazilian population (14.4%). The same frequency of this luteinizing hormone variant in the groups with hypogonadotropic hypogonadism and in the healthy subjects excludes a relationship between this variant and hypogonadotropic hypogonadism.

KEYWORDS: **Luteinizing hormone. Allelic variants of the LH β-subunit gene. Polymerase chain reaction. Brazilian population. Hypogonadotropic hypogonadism.**

Luteinizing hormone (LH), as are other members of the glycoprotein family, is a heterodimer that consists of a common α-subunit and a unique β-subunit that confers biological specificity for the hormone receptor in the target organ.[1] It is produced and secreted in the anterior pituitary, and it is essential in the stimulation of follicle growth and maturation of the oocyte. It has a central role in promoting spermatogenesis and ovulation by stimulating the testes and ovaries, respectively, to synthesize steroids.[1-3] An intact β-subunit is required for its biological activity. However, microheterogeneity and genetic vari-

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ants of LH are known to exist.[4-6] The gene that encodes the human LH β-subunit has been cloned and sequenced;[7] it is located on chromosome 19.[8] Some point mutations that cause hypogonadotropic hypogonadism[9-10] and infertility in both sexes have been identified in the LH β-subunit gene.[11-14]

Two successive point mutations in exon 2 of the LH β-subunit gene cause an immunologically anomalous LH by replacing 2 amino acids in the hormone molecule: Trp8 (TGG) by Arg8 (CGG) and Ile15 (ATC) by Thr15 (ACC).[6] The latter amino acid substitution introduces an extra glycosylation signal into the LH beta chain (Asn13-Ala14-Thr15). This molecular change results in nonmeasurable LH levels when immunoassays using antibodies against the intact LH molecule are used.[6,15-17] These polymorphisms are in complete linkage disequilibrium, and they have been found in both infertile patients and reproductively healthy subjects of several populations, with a relatively high frequency (41.9% to 53.5%) in Finnish Lapp populations and in Australian aborigines.[13-14,18-27] Latin-American populations have not been studied to date.

Our aim was to establish the frequency of the allelic variant Trp8Arg/Ile15Thr in the LH β-subunit gene in a Brazilian population of healthy subjects and in patients with hypogonadotropic hypogonadism (HH).

SUBJECTS AND METHODS

Subjects

This study was approved by the Institutional Review Board of the Hospital das Clinicas, School of Medicine, São Paulo University, Sao Paulo, Brazil.

The study sample consisted of 202 Brazilian adults (115 women) with normal sexual function and 48 patients (24 women) with HH without steroid intake.

DNA Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by standard techniques. DNA amplification of the LH β-subunit gene was carried out using the polymerase chain reaction (PCR) with specific primers: LH forward (ACCTGAACCACACCCACTTC) and LH reverse (GTATGTGTGGTTGCCCTGAG). The PCR procedure was carried out in a total volume of 50 μL reaction mixture, containing 100 to 500 ng of genomic DNA, 30 pmols of each primer, 1.5 mM $MgCl_2$, 200 μM dNTP, and 2.5 U *Taq* polymerase (Amersham Pharmacia Biotech). After the initial denaturation for 5 min at 95°C, samples were subjected to 40 cycles of 1 min at 95°C, 30 sec at 61ºC annealing temperature, 3 min at 72°C extension, followed by a final extension step of 10 min at 72°C.

The PCR products were submitted to digestion with the restriction enzymes *Nco I* for detection of the Trp8Arg point mutation and with *Fok I* for detection of the Ile15Thr point mutation, according to the manufacturer's protocol (New England Biolabs, USA). The *Nco I* digestion of PCR products produced 3 bands of 1050, 575, and 100 bp in TT homozygous subjects, 2 bands of 1150 and 575 bp in CC homozygous subjects, and 4 bands of 1150, 1050, 575, and 100 bp in heterozygous subjects. The PCR products digested with *Fok I* produced 9 bands of 500, 392, 304, 195, 108, 71, 43, 40, and 11 bp in TT homozygous subjects; 8 bands of 500, 435, 304, 195, 108, 71, 40, and 11 bp in CC homozygous subjects; and 10 bands of 500, 435, 392, 304, 195, 108, 71, 43, 40, and 11 in heterozygous subjects. The point mutations were confirmed by direct sequencing using an ABI PRISM 3100 Genetic Analyzer automatic DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA).

Hormonal assays

Basal LH, FSH, estradiol, and testosterone levels were measured in 37 healthy subjects (21 women with chronological age (CA) of 35.4 ± 6.9 years and 16 men with CA of 33.6 ± 12.6 years) and in 27 HH patients (13 women with CA of 19.0 ± 7.3 years and bone age (BA) of 11.9 ± 3.4 years, and 14 men with CA of 20.5 ± 4.0 years and BA of 14.1 ± 2.0 years).

Serum LH and FSH concentrations were determined by commercial, solid phase, 2-site fluoroimmunometric assay (AutoDELFIA hLH Spec and AutoDELFIA hFSH Spec, Wallac Oy, Turku, Finland), based on the direct sandwich technique. This assay uses 2 monoclonal antibodies against the LH β-subunit and should detect this LH variant.

Estradiol and testosterone were measured by commercial solid phase fluoroimmunoassay (AutoDELFIA Estradiol and AutoDELFIA Testosterone, Wallac Oy, Turku, Finland) using polyclonal antibodies. Normal reference values for gonadotropins, testosterone, and estradiol were based on previously reported data.[28]

Statistical analysis

The data are expressed as mean ± SD. The genotypic and allelic frequencies of the LH variant between normal and HH groups were analyzed with the use of the chi-square test and a $P \leq .05$ was considered statistically significant.

RESULTS

The allelic variant Arg8/Thr15 of LH β-subunit gene was found in 29/202 (14.4%) of the healthy subjects and 8/48 (16.6%) of the patients with HH. The frequency of the C allele was not significantly different ($P = 0.996$) between healthy subjects and patients with HH (Table 1).

Table 1 - Allele frequency of the variant Trp8Arg/Ile15Thr of the β-subunit gene in the Brazilian population of healthy subjects and in patients with hypogonadotropic hypogonadism (HH)

Population	LH-β genotypes			C allele Frequency	n
	TT	TC	CC		
Healthy subjects	173	27	2	0.076*	202
HH patients	40	8	0	0.083*	48

T: wild-type allele; C: variant allele
* $P = 0.996$

There was no effect of the allelic variant of the LH β-subunit gene on LH levels in healthy subjects compared to LH levels in patients with hypogonadotropic hypogonadism (HH) (Table 2).

DISCUSSION

There have been speculative suggestions that the allelic variant Arg8/Thr15 of the LH β-subunit gene may correlate with the changes in the pituitary-gonadal function[13,18,19,22] and menstrual disorders.[14,26] Our study indicates that the allelic variant Arg8/Thr15 of the LH β-subunit gene is a polymorphism that is also commonly found in the Brazilian population. The frequency of this allelic variant in the group with hypogonadotropic hypogonadism (16.6%) was similar to that of the healthy subjects (14.4%), excluding a relationship between the variant and hypogonadism.

This allelic variant is not detectable by immunoradiometric assay using highly specific monoclonal antibodies against the intact LH molecule.[6,15-17] This possibility should be kept in mind when inappropriately low levels of gonadotropins are detected in routine diagnostic tests with this kind of assay. In our study, using specific immunofluorometric assay with 2 antibodies against the LH β-subunit, no interference was observed on LH levels in the control group, nor in the HH group.

In conclusion, the allelic variant Trp8Arg/Ile15Thr of the LH β-subunit is a common polymorphism in several populations including Brazilians. The similar prevalence of this allelic variant in both HH and healthy subject groups excludes a role of this variant in the etiology of HH.

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Table 2 - Mean values of LH, FSH, and estradiol or testosterone and allelic variant Trp8Arg/Ile15Thr of the LH β-subunit gene in normal and patients with hypogonadotropic hypogonadism (HH)

Group	Genotype	Men				Women			
		n	LH (U/L)	FSH(U/L)	T(ng/dL)	n	LH (U/L)	FSH (U/L)	E2 (pg/mL)
Normal	TT	15	3.5 ± 1.1*	3.6 ± 2.3	492 ± 168	18	5.2 ± 4.2	6.2 ± 1.8	52 ± 36
	TC	1	3.5	3.1	334	3	4.4 ± 2.0	5.9 ± 2.1	28 ± 4
HH	TT	12	0.62 ± 0.04	1.17 ± 0.35	19 ± 9	12	0.61 ± 0.02	1.18 ± 0.27	<13
	TC	2	0.6	1.05 ± 0.07	<14	1	0.6	1.0	<13

* mean ± SD; T: wild-type allele; C: variant allele

RESUMO

Berger K, Billerbeck AEC, Costa EMF, Carvalho LS, Arnhold IJP, Mendonça BB. Freqüência da variante alélica (Trp8Arg/Ile15Thr) do gene do hormônio luteinizante em um grupo de brasileiros saudáveis e pacientes portadores de hipogonadismo hipogonadotrófico. Clinics. 2005; 60(6):461-4.

OBJETIVO: Avaliar a freqüência da variante alélica (Trp8Arg/Ile15Thr) do gene da subunidade β do hormônio luteinizante em um grupo de brasileiros saudáveis e em pacientes portadores de hipogonadismo hipogonadotrófico. **CASUÍSTICA E MÉTODOS:** Duzentos e dois adultos (115 mulheres) com função sexual preservada e 48 pacientes (24 mulheres) portadoras de hipogonadismo hipogonadotrófico foram submetidos a estudo molecular utilizando técnicas de reação em cadeia da polimerase seguida por digestão enzimática com as enzimas de restrição *Nco I* (para detecção da mutação pontual Trp8Arg) e *Fok I* (para detecção da mutação pontual Ile15Thr). Os níveis basais de hormônio luteinizante e FSH, testosterona ou estradiol foram dosados em 37 indivíduos normais (21 mulheres) e 27 pacientes portadores de hipogonadismo hipogonadotrófico (13 mulheres) pelo método imunofluorométrico (hLH-Spec and hFSH-Spec, AutoDELFIA, Wallac Oy, Turku, Finland).
RESULTADOS: A variante alélica (Arg8/Thr15) do gene da subunidade β do LH apresentou freqüência similar nos indivíduos saudáveis (14.4%) e nos pacientes portadores de hipogonadismo hipogonadotrófico (16.6%). Não houve interferência da variante alélica do gene da subunidade β do LH nos níveis de LH dos indivíduos normais e dos pacientes portadores de hipogonadismo hipogonadotrófico.

CONCLUSÃO: Este estudo indica que a variante alélica Arg8/Thr15 do gene da subunidade β do LH é um polimorfismo comum na população brasileira (14.4%). A freqüência similar dessa variante em indivíduos saudáveis e em portadores de hipogonadismo hipogonadotrófico exclui o papel da variante na etiologia do hipogonadismo hipogonadotrófico.

UNITERMOS: **Hormônio luteinizante. Variantes alélicas do gene da subunidade β do LH. Reação em cadeia de polimerase. População brasileira. Hipogonadismo hipogonadotrófico.**

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