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Genetic Variant of Luteinizing Hormone: Impact on Gonadal Steroid Sex Hormones in Women

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Summary

A common variant of the LHβ subunit has a varying prevalence in various ethnic groups. The consequences of the presence of mutated luteinizing hormone (LH) concern borderline alterations in pituitary/gonadal function that could be mediated by an altered action of variant LH on gonadal steroidogenesis. A comparison of plasma concentrations of gonadal steroid sex hormones was completed in women heterozygous for variant LH and in women with the wild type of LH in three different age ranges. The sample was a randomly selected group of 177 normal women 16 to 72 years old. Variant LH was determined by immunofluorimetric methods using two combinations of monoclonal antibodies. The ratios of LH measured by the two assays indicated whether the subject was wild type homozygote, heterozygote or homozygote for the variant LHβ allele. The carriers of the variant LH allele in the group of postmenopausal women showed higher serum testosterone levels than those with the wild type LH. This is in agreement with the clinical observations made previously showing a slightly higher androgenic action in the population with variant LH. No differences were detected in serum LH, FSH, epitestosterone and sex hormone binding globulin (SHBG).

Key words
LH-variant • Testosterone • Postmenopausal women

Introduction

Some pathological states of pituitary/gonadal functions have recently been found to be due to mutations of the gonadotropin or gonadotropin receptor genes. Although these conditions are extremely rare, they are very informative, elucidating some less characterized aspects of normal gonadotropin function and the molecular pathogenesis of disturbances in sexual differentiation and fertility. In contrast, there is a common polymorphism in the luteinizing hormone (LH) beta-subunit gene, where two point mutations cause two alterations in the amino acid sequence (Trp8 → Arg and Ile15 → Thr) and introduce an extra glycosylation signal to Asn13 (Nilsson *et al.* 1998, Huhtaniemi and Pettersson 1999). The variant form of LH has a world-wide distribution, with a frequency of 0 to over 50 % in various populations (Huhtaniemi *et al.* 1999). In the authors' region, the carrier frequency of LH-variant was 12.2 % and 20.6 % in males and females, respectively (Stárka *et al.* 1999a,b). The carriers of this variant gene are apparently healthy, but certain mild differences in their gonadal function have been found, as reflected by alterations in gonadal steroidogenesis, pubertal

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development and predisposition to diseases such as infertility and the polycystic ovarian syndrome (Tapanainen *et al.* 1999). In older healthy men (aged 73-94 years), a difference in biological response between the two LH forms was suggested pertaining to a difference in the LH vs. fat mass and LH vs. leptin relationships between heterozygote LH variant and wild-type LH subjects (Van Den Beld *et al.* 1999).

The variant LH molecule has increased intrinsic bioactivity *in vitro*, but decreased circulatory half-life *in vivo* (Huhtaniemi *et al.* 1999), and the v-LH promoter is about 50 % more active in cell line transfections than that the wild-type (wt) LH. These differences in LH synthesis and action in individuals homozygous or heterozygous for the v-LH allele are reflected by altered dispositions of pituitary/gonadal function to some of the above mentioned pathological states (Huhtaniemi *et al.* 1999, Huhtaniemi and Pettersson 1999).

These effects could modify the typical influence of LH on steroidogenesis. In conclusion, variant LH may partly explain the large interindividual variations in pituitary/gonadal function, and be a contributing factor in various pathologies of gonadal steroidogenesis. For this reason it would appear to be of interest whether the variant LH in women is associated with changes in the spectrum of gonadal steroid hormones as compared with the normal female population.

Methods

Subjects

A total of 177 females in the age range 16-72 years, with an average age of 41.8 and a median age of 44, was randomly selected from the population register in the Cheb district of West Bohemia. The examination of the population was a part of a survey of iodine deficiency and the probands were examined mainly with respect to their thyroid status, including physical, laboratory and sonographic examinations. Blood was drawn from the cubital vein and serum was frozen until processed in the laboratory. All samples from pregnant women were excluded from the study material. The first group included women in the fertile age range from 16-40 years. The second consisted of women around the menopausal age range between 41-50 years, and the third comprised postmenopausal women over the age of 50.

Immunofluorimetric assays of serum LH

For LH determination the method of Pettersson *et al.* (1992) was used. A Delfia LH Spec kit (Wallac OY, Turku, Finland), which uses two LH β-subunit specific monoclonal antibodies (mAb), served as the reference method assay (Assay 2). In another assay (Assay 1) the capture mAb recognizes a conformational epitope present in the intact α/β LH dimer but not in the subunits, and the detection mAb recognizes an epitope in the α-subunit (Pettersson *et al.* 1992). The assay procedures for the immunofluorimetric assays were followed according to the kit instructions for LH Spec. The ratio of LH values measured by the two assays (Assay 1 and Assay 2) was used to assess the variant and the wild-type LH status. Three separate categories of this ratio were obtained: A) normal ratio >0.9, B) low ratio 0.2 to 0.9, and C) zero ratio <0.15; an individual with a normal ratio has two wild-type LH alleles, a low ratio individual is heterozygous for the LH variant allele, and an individual with a zero ratio is homozygous for the variant LHβ gene, as was confirmed by DNA analysis (Nilsson *et al.* l997, 1998).

The sensitivity of the two immunofluorimetric assays was 0.05 mIU/ml, and the intra-assay and inter-assay coefficients of variation were <4 % and <5 %, respectively, at LH concentrations at or above the lowest standard concentration (0.6 mIU/ml of the WHO International Reference Preparation 80/552).

FSH was measured using a RIA kit from Immunotech (Marseilles, France). Testosterone was determined by standard RIA using anti-testosterone-3-carboxymethyloxime:BSA antiserum and testosterone-3-carboxymethyloxime-tyrosylmethylester-[^{125}I] as a tracer, The intra-assay and inter-assay coefficients of variation were 7.2 % and 10 %, respectively, and the sensitivity was 0.21 nmol/l. Epitestosterone was measured according to Bílek *et al.* (1987). SHBG was measured with the IRMA kit from Orion (Espoo, Finland). The analyses were carried out using a Stratec automatic analyzer from Immunotech (Marseilles, France).

Statistical analysis

The differences between female wild type-homozygotes and LH-variant heterozygotes within the age groups were evaluated by Student's t-test. Given their skewed distribution, the original data underwent power transformation prior to the test.

Results

Significantly higher testosterone levels were found in postmenopausal LH-variant heterozygotes, while in fertile and perimenopausal women the levels

were not significantly different in LH-variant heterozygotes from wild type homozygotes. The differences between wild type homozygotes and heterozygotes in epitestosterone, LH, FSH and SHBG did not reach statistical significance (Table 1).

A weak but significant negative correlation ($r = -0.2796$, n=128, $p<0.01$) was observed in wild type homozygotes between testosterone and age, while no correlation was detected in heterozygotes. The correlations were non-significant in both groups of postmenopausal women. In both wild type homozygotes and heterozygotes a significant negative correlation was detected between epitestosterone and LH ($r = -0.306$, n=130, $p<0.001$ and $r = -0.559$, n=32, $p<0.01$, respectively), as well as in epitestosterone and FSH ($r = -0.441$, n=126, $p<0.0001$ and $r = -0.591$, n=33, $p<0.001$, respectively). However, the correlation was more pronounced in heterozygotes. The most prominent correlations of epitestosterone with gonadotrophins were found in the subgroup of postmenopausal heterozygotes ($r = -0.800$, n=9, $p<0.05$ in LH, $r = -0.700$, n=9, $p<0.05$ in FSH), while no correlations could be detected in age-matched wild type homozygotes.

Table 1. Comparison of serum testosterone, epitestosterone, LH, FSH and SHBG between "wild type" homozygotes and heterozygous carriers of variant allele of LH in women.

Age (years)	**Statistics**	**Testosterone (nmol/l)**		**Epitestosterone (nmol/l)**		**LH (nmol/l)**		**FSH (nmol/l)**		**SHBG (nmol/l)**	
		variant	**wild type**	**variant**	**wild type**	**variant**	**wild type**	**variant**	**wild type**	**variant**	**wild type**
16-40	*n*	15	44	15	52	15	53	15	46	14	47
	Average	1.84	2.18	1.43	1.32	4.9	6.5	7.3	5.2	86.5	100.1
	SEM	0.14	0.12	0.06	0.04	0.9	1.1	2.3	0.7	15.7	8.1
	Median	1.78	2.30	1.50	1.34	3.9	4.5	5.1	3.6	67.1	84.6
41-50	*n*	11	39	11	38	11	41	11	37	11	38
	Average	1.23	1.49	1.26	1.25	16.0	15.0	43.2	30.3	76.3	70.9
	SEM	0.17	0.10	0.10	0.05	5.2	2.4	14.6	6.3	15.5	5.9
	Median	1.08	1.35	1.32	1.32	6.5	8.0	13.5	10.2	60.8	59.5
Over 50	*n*	8	44	8	42	9	44	7	42	8	43
	Average	1.83**	1.49	0.81	0.86	32.3	28.0	93.8	86.8	67.2	63.6
	SEM	0.10	0.09	0.10	0.04	5.4	2.2	16.5	7.6	17.2	6.0
	Median	1.84	1.45	0.79	0.87	31.6	24.5	89.8	74.2	37.2	59.6

*Student's t-test after transformation of the original variables to minimum skewness was used for the evaluation of the differences between homozygotes and heterozygotes in the levels of hormones.** significantly different (p<0.01) from wild type homozygotes, n – number of subjects.*

Discussion

It is possible that the differences between the two LH forms are due to the biological response in representative groups of healthy elderly men (Van Den Beld *et al.* 1999) and in women with the polycystic ovary syndrome (PCOS) (Tapanainen *et al.* 1999). In men, the testosterone levels and the degree of reported frailty did not differ between the wild-type LH group and the heterozygote LH variant group. In women with the polycystic ovary syndrome (PCOS), the lower incidence of v-LH in obese PCOS patients suggests that v-LH somehow protects obese women from developing symptomatic PCOS. Nevertheless, regional differences in this finding between patients with apparently similar diagnostic criteria emphasize the multifactorial nature of this syndrome. The relation of the LH variant to levels of steroid sex hormones has not previously been studied. It

follows from the data provided by the present study that there exists a tendency to higher androgen levels in older women heterozygous for LH but not in other conditions regulating the androgenic status, such as SHBG. This may be of particular interest in the light of the data of Kero *et al.* (2000) reporting the role of long-term gonadotropin effect on adrenal steroidogenesis. On the other hand, the ovaries of postmenopausal women consist primarily of stromal cells chiefly producing testosterone and only lesser amounts of other androgens and estradiol (Nagasako 1983, Judd and Fournet 1994, Ushiroyama and Sugimoto 1995, Longcope 2001). Variant-LH exhibits higher biological activity, although its circulatory half-life is shorter (Takahashi *et al.* 2000, Lamminen and Huhtaniemi 2001). We could speculate whether the higher occurrence of v-LH could eventually be associated with higher incidence of stromal hyperplasia that is characterized by elevated production of testosterone and by unchanged production of estradiol (Manieri *et al.* 1998, Sluijmer *et al.* 1998).

A remarkable difference exists in correlation of the level of endogenous antiandrogen epitestosterone and gonadotropins between postmenopausal heterozygotes and age-matched homozygotes with wild type LH for the LHβ subunit. This finding could be of importance in connection with the antiandrogenic effect of epitestosterone (Stárka *et al.* 1989, 1991) that inhibits several enzymes on the metabolic pathway to androgens (Stárka *et al.* 1989, 1991, Bičíková *et al.* 1992, 1993a) and influence the levels of gonadotropins (Bičíková *et al.* 1993b). The response of steroidogenesis to the two LH forms exhibits a difference the biological role of which needs further investigation.

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